Alexis Labhart

Clinical Endocrinology

Theory and Practice

With a Foreword by George W. Thorn

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With 400 Figures

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Foreword

Periodically in the evolution of an important branch of clinical medicine there develops a critical need for a textbook which combines with the clinical aspects of disease syndromes an in-depth review of the sciences basic to the disorders discussed, as well as a carefully selected but comprehensive review of pertinent literature. LABHART's *Clinical Endocrinology* revised and translated into English provides for this need in the field of endocrinology in an exemplary manner.

Prof. LABHART has selected his individual authors with great care, and they in turn have provided authoritative monographs. An interesting, useful and informative introduction to each chapter is provided by a tabulation of the dates of important or significant contributions to the field. The chapter subdivisions present in great detail a wide variety of subjects such as embryology, anatomy, biochemistry, physiology, individual hormones and their analogues, biosynthesis, metabolism and regulation of hormone release as well as a full discussion of the clinicopathological correlations. The bibliography is unusually extensive and will provide an important source book for all investigators and students in the field.

It is a tremendous accomplishment that a work of this magnitude, so broad and so extensive, could be brought up to date in its English translation with such a relatively short lag period between the author's revision and the date of publication. This volume will fill an important place in medical libraries as well as providing experts in the field with authoritative source material. Furthermore, in these days of shortened medical school curriculum in the basic sciences the sections in the book concerned with such areas as anatomy, biochemistry and physiology will provide students with an excellent background for their clinical responsibilities.

The translation of a textbook of this magnitude presents a formidable undertaking. Prof. LABHART and his co-authors are to be congratulated for making available to the English literature this updated version of their classic.

Boston, April 1974 GEORGE W. THORN

Preface to the 2nd German Edition

There has been such rapid development in the background knowledge basic to clinical endocrinology that for the second edition everything except the clinical descriptions had to be completely rewritten. Two of the authors involved in the first edition, Prof. K. G. OBER and Prof. T. ZANDER, were unable to contribute to this edition due to new commitments. Our gratitude is due to Prof. W. E. SCHREINER and Dr. P. J. KELLER, under Prof. E. HELD at the Zurich School of obstetrics and gynecology, who took over and rewrote the chapters on ovary and pregnancy.

Despite the enormous increase in the content, it has been possible to keep the number of pages about the same as in the first edition and avoid the necessity of a two-volume work by using a larger format and omitting many illustrations we had planned to include. We had intended to reproduce a large number of electron micrographs but have had to content ourselves with citing the original papers.

Where it was only possible to refer very briefly to newly discovered facts the original papers are cited. They are quoted in the text by first author and year of publication only, to save space. The lists of references for each chapter have again been divided up into subsections, and they also contain publications not referred to in the text. They can thus be used independently. The publications in the list are a personal selection; preference is given to the most recent reviews and we lay no claim to completeness or citation of first descriptions. Early papers of outstanding significance have been retained in the lists of references alongside the most recent publications on the same subjects.

Almost 20 years ago, when I asked my clinical teacher Prof. SCHÜPBACH, whether he thought I might venture to write a textbook on the total field of endocrinology, he thought for some time before answering in the affirmative, and followed up this reply with the observation that the second edition might be successful. It is not for us to judge whether this has been borne out. However, if we succeed in providing endocrinologists and other specialists with a useful survey of the whole field of clinical endocrinology and the nonclinical disciplines essential to its comprehension before it is broken down into subspecialties, we shall feel we have attained the goal of the second edition.

We are much indebted to Springer-Verlag, to Dr. H. GÖTZE for his kindly advice and unfailing help in the work of planning, and to Mrs. T. DEIGMÖLLER for her tireless help in the meticulous work involved in the production of the second edition. We should like to thank our secretaries, Mrs. WÄCHTER-BRANDENBERG, Miss L. WAGNER, Miss M. SCHITTENHELM, and Miss M. WALDVOGEL, and the librarians Miss F. BELART and Miss S. DOMEISEN for their help, our scientific assistant Dr. K. BAUMANN for the compilation of the subject index, Dr. J. ZAPF and Dr. U. KELLER for correction of the proofs, and numerous of our colleagues for constructive criticism and advice.

Zurich, April 1971 A. LABHART

Preface to the 1st German Edition

There has been substantial progress in endocrinology over the past 20 years, and the specialty's position within medicine as a whole has changed radically.

The science referred to as endocrinology 20 years ago was concerned predominantly with description and morphology; with the realization that hormones were involved in the regulation of metabolism it has become an integral element of our knowledge of metabolic disorders, and as a result of the discovery of the effects of adrenal hormones on inflammation and the involvement of the pituitary-adrenal axis in resistance mechanisms there is now hardly a single field of medicine that is not bound up with the problems of endocrinology.

During the war years and the period immediately after the war, progress was particularly rapid. Biochemically oriented medicine was undeniably more advanced in the United States than in Europe, where the tradition of medicine based on morphology and bedside observation prevailed; we have attempted to achieve a blend of both these elements in this book.

We were fortunate to have the opportunity of observing American methods of clinical medicine and research during extended stages of study at clinical endocrinology centers in the United States Peter Bent Brigham Hospital, Boston (G. W. THORN), Johns Hopkins Hospital, Baltimore (L. WILKINS), Mayo Clinic, Rochester (R. M. WILDER), University of California medical school (H. D. MOON), and we have kept in close contact and followed developments in America ever since.

Four of the authors (COURVOISIER, HEDINGER, LABHART, and WERNLY) are former students of the late Prof. SCHÜPBACH (Berne), who always kept up to date with the latest developments in endocrinology and had the ability to introduce them into bedside medicine. It was he who first aroused our interest in endocrinology. He took a lively interest in the progress of this book right up to his death.

The idea of writing a textbook on endocrinology was initially proposed by Prof. P. H. ROSSIER. His medical clinic in Zurich, as a site of both day-to-day medical activity and experimental medicine, was well suited to the blend of theory and practice we have tried to achieve. We have tried to adopt the same functional approach to medicine as Prof. ROSSIER. We would not have ventured to embark on this work without his initiative and encouragement.

The kindness of Prof. G. FRANCONI and Prof. C. KAUFMANN made it possible to include pediatric and gynecological endocrinology, without which the work would have been incomplete. We are indebted to Prof. UEHLINGER of Zurich for his valued and unstinting advice and his untiring help in the selection and acquisition of the illustrations, and no less to Springer-Verlag, Heidelberg, for their unfailing help in the planning and production of our textbook.

Zurich, December 1956 A. LABHART

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I. General Aspects of Endocrinology

HANS BÜRGI

A. Introduction

Hormones are chemical messengers which are dissolved in extracellular fluid and carry information between cells*. Within any single cell, information is transmitted through chemical compounds in a similar manner. Such intracellular messengers include enzymes, allosteric ligands and proteins, certain ribonucleic acids and deoxyribonucleic acids. It is noteworthy that deoxyribonucleic acids can also store information, a feature which is lacking in the endocrine system. The function of hormones might be compared to that of nerves, which also convey information from one cell to another.

Intercellular communication cannot always be definitely assigned to either the neural or the hormonal pathway. There are certain cases where the secretion of hormones is influenced by neural stimuli, and other cases where hormones can become direct transmitters of neural information, e.g. norepinephrine, which is secreted at the nerve endings of the sympathetic nervous system. The neurosecretion of the supraoptic and paraventricular nuclei of the diencephalon is a mechanism having characteristics of both neural and hormonal communication. In this case, information flows along the axis cylinder of a nerve cell in the form of discontinuous signals. These signals are not

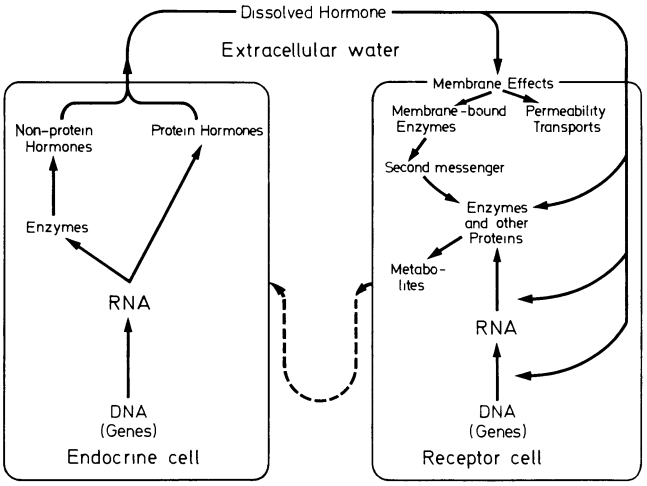


Fig. 1. Schematic representation of the flow of information within an endocrine cell and from there to the receptor cell. The dashes indicate a feedback loop. Abbreviations: RNA: ribonucleic acid; DNA: deoxyribonucleic acid

In contrast to the endocrine system but like the intracellular genetic system, the nervous system is also capable of storing information. variations in electrical potential but rather hormone-containing particles which are converted to soluble hormones in the posterior lobe of the pituitary gland. Hormone molecules are dissolved in the extracellular fluid and travel from their endocrine cell of origin to their appropriate receptor or target cells. Usually they are carried by the bloodstream and quickly reach their target cells, which may be quite

* This definition is based on physiological considerations and does not include hormones or their pharmacological derivatives as used for therapeutic or diagnostic purposes. A similar "operational" definition of hormones has recently been exhaustively discussed by VALLOTTON (1969).

distant. Hormones which diffuse only locally in the interstitial fluid surrounding an endocrine cell are called *tissue hormones*.

Hormone molecules transfer their information through contact with a receptor at the target cell. They thereby regulate certain chemical or physical processes within the receptor cell which finally result in known biochemical and clinically evident hormonal actions. In many cases the information contained in the hormone is reconverted at the cell membrane into an intracellular second messenger which in turn controls a process within the cell. In this manner information issued by the endocrine cell eventually reaches its destination. However, the flow of information in the organism does not cease at this point. Processes may be triggered in the target cell which lead to the formation of feedback loops. Under the influence of a hormone a receptor cell may release greater or smaller amounts of a chemical compound (e.g. a metabolite or another hormone) into the extracellular fluid, hereby informing the original endocrine cell that it has received and executed the instruction. Frequently, in a negative feedback, this will inhibit the endocrine cell from issuing the same information, i.e. from secreting more hormone. Fig. 1 shows a simplified representation of the flow of information from the endocrine cell to its receptor and back to the endocrine cell of origin. The individual steps in the transfer of information by hormones will now be discussed in detail and illustrated by typical examples.

B. Endocrine Genetics

Hormones are of great interest to the geneticist primarily because certain diseases are known to be caused by inherited deficiencies of specific hormones. Examples among the protein and peptide hormones are the inherited deficiencies of antidiuretic hormone and of growth hormone. Whether diabetes mellitus is due to an inherited disorder of insulin biosynthesis is still controversial. In addition, several endocrine disorders are caused by an inherited deficiency of the enzymes necessary for the biosynthesis of non-protein hormones. Examples are the various adrenogenital syndromes and certain forms of congenital hypothyroidism. Inherited failure of the target organ to respond to its corresponding hormone is the cause of pseudohypoparathyroidism and renal diabetes insipidus.

Patients with the syndrome of multiple endocrine adenomata suffer from an inherited tendency to develop tumors in various endocrine glands. Certain disorders of the thyroid gland, such as thyrotoxicosis, Hashimoto's thryoiditis, and primary myxedema, all of which appear to be associated with autoimmune processes, show a familial incidence without clear-cut genetic transmission.

Other endocrine diseases, for example, Turner's syndrome and Klinefelter's syndrome, are due to chromosomal disorders. These syndromes have provided important information regarding the function of sex chromosomes.

It may be of interest to the biochemically oriented geneticist to know that hormones were among the first proteins to have their amino acid sequences fully elucidated. Surprisingly, certain hormones with very different actions were found to be chemically closely related. Thus, large parts of the ACTH molecule are identical to the α-MSH molecule and a whole subunit of the TSH molecule is identical with a subunit of the LH molecule. Such close similarity between two proteins is probably the end result of gene duplication, an important mechanism by which new proteins are acquired in the course of evolution.

C. Biosynthesis of Hormones

Research on the biosynthesis of protein hormones has long been hampered by the lack of suitable isolated tissue preparations and, in particular, of corresponding cell-free systems. However, one may assume that protein hormones are synthesized at the ribosomes according to the "classic" concepts of molecular biochemistry. Insulin is the protein hormone whose biosynthesis has been most fully studied. The linkage of the two peptide chains of insulin by two disulfide bridges represents a particular biochemical problem. The earlier view that the two protein chains were separately synthesized and subsequently joined by the disulfide bridges no longer seems tenable. The investigations of STEINER (1967) in both man and rat demonstrated a single-chained insulin precursor – proinsulin – containing both the *A* and the *B* chains of insulin. The amino acid sequence of proinsulin has been worked out recently by CHANCE (1968). The two-chained insulin molecule arises through enzymatic removal of a peptide, the connecting or *C*-peptide, from the proinsulin molecule. The mechanism suggested by STEINER and CHANCE for the biosynthesis of insulin is shown in Fig. 2. Whether analogous "prohormones" also exist for other protein hormones remains to be investigated. Strictly speaking, thyroglobulin could be considered the prohormone of thyroxine, since thyroxine is an

amino acid which is synthesized as a component of the large protein thyroglobulin. Hormone secretion involves the splitting off of thyroxine from thyroglobulin by proteolysis. It is not known whether the low molecular weight peptide hormones of the posterior pituitary lobe are

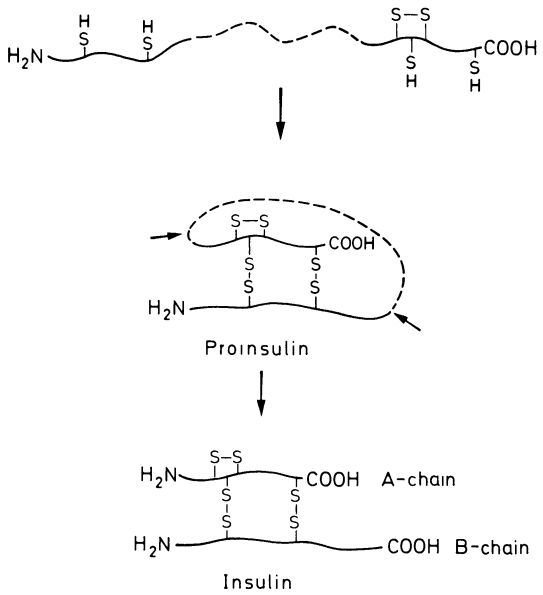


Fig. 2. Biosynthesis of insulin according to D. F. STEINER (1967). The dashed portion of pro-insulin, the so-called connecting peptide, is split off enzymatically at the points indicated by arrows

also synthesized via a prohormone at the ribosomes. It is theoretically conceivable that, like certain bacterial peptides, they are synthesized via an enzymatic non-ribosomal pathway (MACH, 1963), or they may be split off from larger prohormone molecules. The biosynthetic mechanisms for the nonprotein hormones (steroids, catecholamines) have been fully elucidated and will be discussed in the appropriate sections.

D. Storage and Release of Hormones

Hormones of the pancreatic islets, anterior and posterior pituitary gland, adrenal medulla, and thyroid gland are not only synthesized but may also be stored in considerable amounts within the endocrine organ. In contrast, steroid hormones of the adrenal cortex and gonads are not stored in any significant amount. Granules surrounded by membranes can be detected in most of the endocrine cells which do store hormones by light and electron microscopy. There is good evidence, sometimes indirect, that the granules contain stored hormone. Catecholamines can be chemically identified in the granules of the adrenal medulla (WURTMAN, 1965), and insulin has been demonstrated by immunofluorescence in the *B*-cell granules of the pancreatic islets. Few data are available on the physicochemical state of stored hormones. Adrenaline and ATP are found in a fixed molar ratio of 4 : 1 in the granules of the adrenal medulla (WURTMAN, 1965). However, the nature of a possible chemical bond between these two substances is not known. Thyroid hormones are stored in an unusual manner, namely extracellularly in the colloid as components of thyroglobulin. Thyroxine and triiodothyronine are probably bound by peptide bonds to this large glycoprotein molecule.

Release implies the expulsion of the hormone from its endocrine cell into the extracellular fluid. We have at present only limited information about the complex process by which hormones, which are often poorly fat-soluble, are transferred through the lipid-rich cell membrane. LACY (1967) was able to show by electron

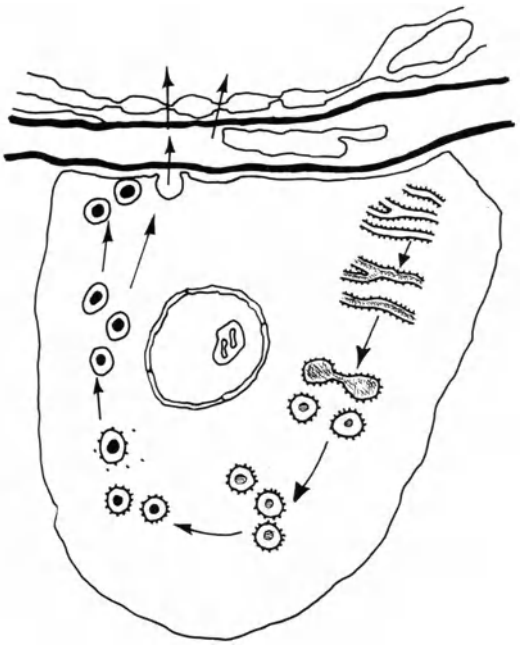


Fig. 3. Biosynthesis and release of insulin. Hormone synthesis begins in the endoplasmic reticulum. The hormone precursor, which is at first still amorphous and biologically inactive, is surrounded by a membrane and is converted into crystalline insulin or pro-insulin. The granular content is forced by emeiocytosis into the interstitial fluid where insulin is dissolved. Finally, the dissolved insulin diffuses into the capillaries. (From P. E. LACY, 1967)

microscopy that insulin granules migrate from the center of the pancreatic *B*-islet cells toward the periphery in the course of insulin secretion (Fig. 3). The granular membrane then fuses with the cell membrane and the granular content is ejected into the extracellular fluid by a process

termed emeiocytosis. According to recent investigations, the granules in the cell move along microtubular structures which may be surrounded by contractile filaments (LACY, 1968). There is now good evidence that adenosine 3′,5′-monophosphate (cyclic AMP) is involved in the mechanism for the release of insulin (MALAISSE, 1967; LAMBERT, 1967). The secretory granules of the anterior pituitary have recently been isolated by differential centrifugation and found to be rich in a protein kinase which, under certain conditions, is dependent upon cyclic AMP and phosphorylates structural proteins of the granule (LABRIE, 1971). This again suggests that cyclic AMP has a role in hormone release.

The process of hormone release must occupy a central position in the transfer of information by hormones, as underscored by the following reasoning: Most hormones have a fixed peripheral disposal rate, or half-life, once they have left the endocrine cell. Therefore, a given hormone concentration in the extracellular fluid which is crucial for the information to be conveyed can only be achieved and maintained by adjusting the rate of hormone release. Consequently, hormone release must be a delicately controlled process.

E. Hormone Transport

As stated initially, hormone molecules travel in the extracellular fluid from their endocrine cell of origin to their receptor cell. In most cases, hormones diffuse from the interstitial fluid surrounding the endocrine cell into the blood, which assures rapid transport to the receptor cell. Tissue hormones are an exception. They diffuse only locally in the interstitial fluid and thus reach only neighbouring cells. Often the concentration of a hormone is not uniform in all parts of the circulation, i.e. the circulating blood is subdivided into hormonal compartments. Such a process is probably of great importance for hypothalamic releasing factors. These factors attain an effective concentration only in the very small pituitary portal veinous system. The effects of glucagon and insulin on the liver are probably influenced by the fact that their concentration is significantly higher in the portal system than in the arterial blood.

Certain hormones are bound to specific proteins in the blood. This is true of thyroxine and cortisol, and probably also of estradiol, progesterone and testosterone. Whether insulin circulates bound to a protein is controversial. Assuming that only *one* single protein with only *one* binding site per molecule combines with the hormone, the reaction occurs according to the general formula:

$$h + p \rightleftarrows hp$$

h = free hormone, p = carrier protein, hp = hormone-carrier protein complex.

Experiments with isotopically labeled hormones have shown that the free hormone is in general fully exchangeable with the protein-bound hormone. This suggests that the hormone binds to the protein by noncovalent bonds and that the free and the bound hormone are in an equilibrium governed by the law of mass action. In the case of thyroxine, peripheral hormone action correlates better with the concentration of free hormone in the plasma than with the concentration of the protein-bound hormone. It has been generally held, therefore, that only free hormone molecules can diffuse from the blood into the interstitial fluid and reach their receptor cells (ROBBINS, 1960). It follows that appropriate clinical assessment of thyroid function would theoretically require the measurement of the free hormone concentration. Unfortunately, in the case of both cortisol and thyroxine, measurements of the free hormone in the plasma – though feasible – are still technically laborious. For both hormones the clinician must generally be satisfied with an estimate of the total hormone. Understanding of the mathematical relationship between the value of total hormone supplied by the routine chemical laboratory and the physiologically important free hormone concentrations is therefore of theoretical as well as clinical importance.

The following derivations can be made from the general formula above:

$$K = \frac{[h] \cdot [p]}{[hp]} \quad \text{(law of mass action).} \tag{1}$$

K = dissociation constant of hormone-carrier complex.

$$[P] = [p] + [hp] \tag{2}$$

= total concentration of carrier protein (free plus bound),

$$[p] = [P] - [hp], \tag{3}$$

$$K = \frac{([P] - [hp]) \cdot [h]}{[hp]} \quad \text{(by substitution of (3) into (1)),} \tag{4}$$

$$= \frac{[P] \cdot [h]}{[hp]} - [h], \tag{5}$$

$$[hp] = \frac{[P] \cdot [h]}{K + [h]}, \tag{6}$$

$$[H] = [h] + [hp], \quad \text{(by definition total amount of hormone: free plus bound),} \tag{7}$$

$$[H] = \frac{[P][h]}{K + [h]} + [h]. \quad \text{(by substitution of (7) into (6)).} \tag{8}$$

It becomes clear from Eq. (8) that the measured total hormone concentration $[H]$ is not a simple straightforward function of the physiologically important free hormone concentration $[h]$. On the other hand provided the free hormone remains constant, $[H]$ is a strict linear measure of the concentration of carrier protein $[P]$. The curves in Fig. 4 illustrate the mathematical relationships derived from Eq. (8). Although the calculations above are based on simplifying assumptions (see below), the curve shown in Fig. 4 resembles that obtained from actual measurements of total and free thyroxine (REICHLIN, 1967).

The assumption that there is only one species of carrier protein for every hormone is probably an oversimplification. Thus, Eq. (8) and the corresponding curves only approximate the actual conditions. Mathematical derivations with more general validity can be found in ROBBINS (1960) and SANDBERG (1966). In fact, thyroxine is bound to three proteins in the plasma, thyroxine-binding globulin (TBG), thyroxine-binding pre-albumin (TBPA) and albumin itself (OPPENHEIMER, 1968). TBG and TBPA have been isolated from the plasma and extensively analyzed. Cortisol is bound to cortisol-binding globulin which has also been termed transcortin. This protein has also been isolated (SANDBERG, 1966). All three hormone-binding proteins so far investigated appear to bind one hormone molecule per protein molecule.

The importance and function of hormone-binding plasma proteins are far from clear. These proteins do not appear to serve the purpose of improving solubility of hormones in the plasma, since cortisol and thyroxine

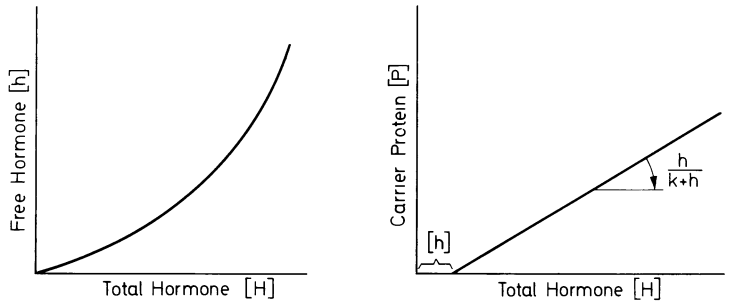


Fig. 4. Theoretic curves of $[h]$ and $[P]$ as functions of $[H]$ in Eq. (6) p. 4. In the curve on the left, $[P]$, the total carrier concentration, is taken to be constant. In the curve on the right, $[h]$, the concentration of free hormone, is taken as a constant. $[H]$ may be considered representative of the total serum hormone (e.g. cortisol or thyroxine)

From Eq. (8) it follows that, in order to calculate the level of free hormone from the measured total hormone, one must also know the concentration of carrier protein $[P]$ and the dissociation constant $[K]$. However, these two parameters are still not generally available to the clinician, and he therefore assumes that both are constant values. This simplification is only justified by the lack of suitable practical methods of measuring the free hormone concentration. One must be aware that, for example, in patients with a low plasma protein level, as in cirrhosis of the liver or the nephrotic syndrome, low plasma cortisol and serum thyroxine concentrations may be measured yet the level of free hormone can be quite normal. The reverse can, of course, be true when the proteins binding cortisol and thyroxine are elevated, a condition frequently encountered today in women taking oral contraceptives.

would be easily soluble in water in the physiologically required concentrations. The possibility that hormone-binding proteins may regulate hormone release to tissues is conjectural. For this to occur, concentrations and dissociation constants of hormone-binding proteins would have to adjust rapidly to different metabolic situations. There is no reliable evidence for such rapid adaptations so far, except perhaps in the case of TBPA, the concentration of which falls in acute inflammatory processes, resulting in a temporary rise of free hormone in the plasma (INGBAR, 1960; OPPENHEIMER, 1963). An important regulatory function of these proteins is refuted by the fact that people with an inherited deficiency of TBG are absolutely healthy and have normal thyroid function. Alternatively, the possibility has often been considered that hormone-binding proteins may prevent or slow down disposal and excre-

tion of their corresponding hormone, thus resulting in more economical hormone secretion. This view is contradicted by the observation that people with inherited TBG deficiency have a normal secretion rate of thyroxine (NICOLOFF, 1964). In conclusion, therefore, we agree with DE MOOR (1968) that the physiological significance of hormone-binding proteins in the plasma is not known.

F. Transfer of "Hormonal" Information to the Receptor Cell

"Hormonal" information in the plasma and interstitial fluid exists in the form of the hormone concentration which may be zero or any chosen positive value. In most cases the receptor cells seem to be sensitive to the hormone concentration in the surrounding extracellular fluid. In many cases the mathematical relationship between hormone concentration and hormone effect obtains according to ARIENS (1956), making the following two assumptions:

1. Hormones act by combining reversibly with cell receptors (see p. 7) according to the general formula:

$$r + h \rightleftarrows rh, \tag{9}$$

where r = receptor, h = hormone and rh = receptor-hormone complex.

In at least one system, the binding of insulin to fat-cell receptors, this assumption has been verified, since both the association and dissociation reaction followed the expected kinetics (CUATRECASAS, 1971 a).

2. Hormone effect is directly (linearly) proportional to the concentration of the hormone-receptor complex:

$$E_h = \alpha[rh], \tag{10}$$

where E_h = action of hormone h, $[rh]$ = concentration of receptor-hormone complex, α is a constant, (proportionality constant).

If $[R] = [r] + [rh]$, the total concentration of receptor (i.e. free receptors plus those bound to hormone), according to the law of mass action, the dissociation constant (K) of the hormone-receptor complex is:

$$K = \frac{[r]\cdot[h]}{[rh]} = \frac{([R] - [rh])[h]}{[rh]}. \tag{11}$$

From this, the concentration of hormone-receptor complex can be calculated as follows:

$$[rh] = \frac{[R]}{\frac{K}{[h]} + 1}. \tag{12}$$

Through substitution in Eq. (10):

$$E_h = \alpha[rh] = \frac{\alpha[R]}{\frac{K_h}{[h]} + 1}. \tag{13}$$

The maximum effect (E_h max) is achieved if all receptors are saturated with hormone, thus if

$$[rh] \rightarrow [R]$$

then

$$E_h \max = \alpha[R]. \tag{14}$$

Substitution of Eq. (14) in Eq. (13) gives:

$$E_h = \frac{E_h \max}{\frac{K_h}{[h]} + 1}. \tag{15}$$

From this equation, which is formally identical with that of MICHAELIS-MENTEN for enzyme kinetics, theoretic concentration-response curves can be drawn. Such a theoretic curve is shown in Fig. 5. It demonstrates that hormone action should be linearly proportional to the *logarithm* of hormone concentration in the middle part of the S-shaped curve.

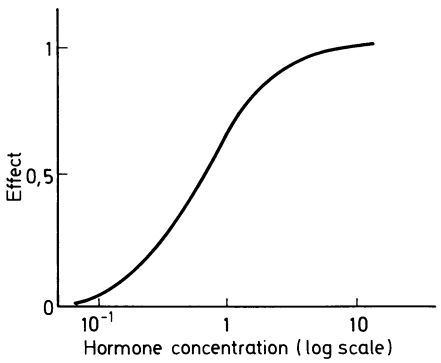


Fig. 5. Relationship between hormone concentration and hormone action. Theoretic curve calculated according to formula 15 on p. 6. (From E. J. ARIENS, 1956)

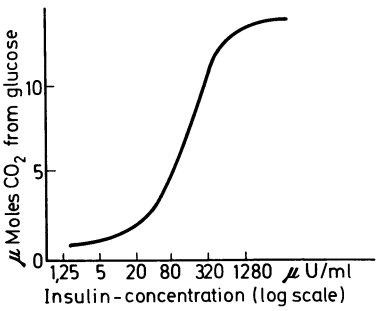


Fig. 6. CO_2 production from glucose in isolated fat cells incubated *in vitro* in the presence of different concentrations of insulin. (From J. GLIEMAN, 1965)

In practical experiments, hormonal effects often do follow curves similar in shape to those calculated theoretically, as seen in Fig. 6. The relationship between hormone concentration and effect derived from Eq. (7) may well be an oversimplification of the actual state of affairs. Recent experiments by RODBELL (1971 a, 1971 c) have shown that in the case of glucagon neither assumption (1) nor assumption (2) strictly hold. Thus, hormone-target cell interactions may be vastly more complicated than outlined above.

Occasionally, the effector cells do not seem to respond to the surrounding hormone concentration but rather to the velocity at which this hormone concentration changes. In mathematical terms, therefore, these effector cells are sensitive to the first differential of hormone concentration against time. CLYNES (1961) showed that a "differential" sensitivity of this kind existed in the pupillary reaction to light stimuli. DALLMAN (1969) found evidence of an analogous phenomenon in an endocrine system, namely the action of corticosteroids on hypothalamic centers.

G. Receptors

For a hormone molecule to produce an effect it must come into direct physical contact with the target cell. It must therefore be accepted that the target cell possesses certain structures termed receptors or discriminators which "recognize" a hormone and convert the information contained in the dissolved hormone into biochemical processes within the cell. During this process of recognition a physical or chemical reaction must take place between the hormone molecule and its receptor. It is likely that this reaction consists of the binding of the hormone molecule to its receptor. The three-dimensional shape of the receptor molecule is probably such that it is complementary to the shape of the hormone molecule or of parts of it, in which case the hormone and the receptor would fit together like pieces of a jigsaw puzzle. Since receptors transmit information from the extracellular fluid into the cell, it is logical to assume that they are located in the cell membrane facing outwards. Experimental support for this view has come from ingenious studies by CUATRECASAS (1969). He incubated isolated fat cells in the presence of insulin which was bound chemically to beads of agarose (a carbohydrate polymer), and found that insulin still exerted its biological effect on glucose transport. Since it is practically inconceivable that the agarose particles penetrated into the cells, the interaction between hormone and receptor must have taken place at the outer face of the cell membrane.

Some very relevant information about the nature of the bonds between hormones and receptors has accumulated in the past 10 years. If it is assumed that the combination is reversible (which is probable but remains to be proven for many hormone-receptor interactions), then noncovalent bonds are the most likely to be involved. On the basis of extensive comparisons of the activity of steroid hormones and their numerous chemical analogues, BUSH (1962) was able to identify certain parts of the steroid molecules which must participate in the attachment to the receptor. One conclusion he reached was that one way glucocorticoids were bound to their receptors was through a hydrogen bond involving the 11β-hydroxyl group of the hormone. Ionic and hydrophobic interactions produce other types of bonds. Hydrophobic bonds develop through the tendency of hydrophobic (uncharged, apolar) groups of molecules to associate in aqueous solution. Such apolar groups are found in abundance in the hydrocarbon side chains of certain amino acids of proteins. The bonds developed in this way are weak, but are of eminent importance, e.g. for the maintenance of the tertiary and quarternary structure of proteins, because of their large number. RODBELL (1971 d) demonstrated that a region rich in hydrophobic amino acid side chains of the glucagon molecule is involved in the binding to liver-cell membranes. The binding can be reversed by 0.4 M urea. Both observations strongly implicate hydrophobic interactions between hormone and receptor. In the case of protein and peptide hormones which contain disulfide bridges (insulin, antidiuretic hormone), the possibility has been considered that covalent bonds may occur with sulfhydryl groups of the receptors through the formation of a mixed disulfide between receptor and hormone. The *in-vitro* action of insulin upon adipose or muscle tissue can in fact be prevented by the use of sulfhydryl-blocking agents such as N-ethyl maleimide (OTT, 1963). However, the conclusion that this proves the formation of a mixed disulfide between insulin and its receptor has been seriously challenged, and excellent evidence has been produced that a disulfide-sulfhydryl exchange is *not* involved in the action of antidiuretic hormone (WALTER, 1967) or in that of insulin (CUATRECASAS, 1971).

BUTCHER (1965) and GRAHAME-SMITH (1967) observed that the maximal effects of epinephrine on adipose tissue and of ACTH on the adrenal cortex were not limited by the number of receptors, as might have been deduced from Eqs. (14) and (15) in the previous section; in

fact these tissues appeared to have an excess of hormone receptors and the maximal hormone effect is limited by secondary processes triggered in the target cell by the hormone. Usually, these secondary processes are chemical reactions where the amount of an enzyme or substrate can be rate limiting factors. ARIËNS (1966) gives a full discussion of the kinetic implications of such spare receptors.

The attachment of a hormone to the target cell can be quantitatively demonstrated by the fact that the concentration of hormone is greater in the cell than in the surrounding fluid. Such an accumulation of hormone in the target cell has been demonstrated for the non-protein hormones aldosterone (SHARP, 1966), estradiol (JENSEN, 1962), thyroxine (HOCH, 1967) and possibly also for cortisol (SEKERIS, 1967). *In vitro*, aldosterone is rapidly and completely bound to toad bladder mucosa within 30 min. It is interesting that hormonal effects become evident only after 40–120 min. The binding occurs at two different receptors with very different affinity constants of 1.4×10^{14} and 4×10^{12} Mol^{-1} (SHARP, 1966). The attachment of aldosterone to the receptors is reversible and 96% of the bound hormone can be eluted from the mucosa in chemically unaltered form. This strongly implies that the bonds between the hormone and its receptors are noncovalent.

JENSEN (1962) injected estradiol labeled with tritium into female ovarectomized rats, and found that the hormone accumulated in the uterus, vagina and anterior pituitary lobe. Fractionation of cells and autoradiographic investigations showed that estradiol was bound predominantly to the cell nuclei (STUMPF, 1966). TOFT (1966) isolated a protein from rat uterus, whose properties fulfilled the criteria of a specific estrogen receptor. JENSEN (1972) found two different estrogen receptors in rat uterus: one was a cytoplasmic protein with a sedimentation constant of 4 S, and the other, present in rate-limiting amounts, a nuclear protein with a sedimentation constant of 5 S. There is good evidence that estrogen binding converts the cytoplasmic receptor from its 4 S to a 5 S form, which then migrates to the nucleus.

Hypothyroid rats bind large amounts of injected thyroxine to their liver mitochondria. Between 1 and 5 molecules of thyroxine per respiratory chain unit are bound during this process (HOCH, 1967).

Injected cortisol is very quickly absorbed into liver cells. A part is metabolized there, whereas a smaller part remains bound to the cell nuclei as unchanged cortisol (SEKERIS, 1967). BAXTER (1972) has shown that in the cytosol of liver cells, cortisol or other steroids with glucocorticoid activity first bind reversibly to a cytoplasmic soluble receptor protein and thereby produce some change in shape (conformation) of this receptor molecule. The protein-steroid complex travels to the nucleus, and, thanks to the small conformational change produced by the binding of the steroid, it can now bind to nuclear DNA and exert control over the transcription of certain genes. The protein receptor alone without steroid hormone cannot bind to DNA because its shape is slightly different and it does not fit the DNA receptor. The details of the following reaction, the selective transcription of certain DNA strands, has not been fully worked out, but the end result appears to be the production of more of the enzyme tyrosine aminotransferase. The complex sequence of events is depicted schematically in Fig. 11 (p. 15). For a better understanding the reader is also referred to the section on protein synthesis (p. 15 and Fig. 10).

Reversible binding of peptide hormones to their target cells has been controversial until recently. Thus CROFFORD (1968) found that insulin disappeared in vitro from an incubation medium containing adipose tissue cells. He attributed this to destruction of insulin and he was unable to detect reversible binding to the fat cells. However, in the past two years excellent evidence has emerged in support of reversible binding of insulin, ACTH and glucagon. CUATRECASAS (1971c) has shown that ^{125}I-labeled insulin binds reversibly to fat cells. The insulin could be recovered unchanged from the cells by elution. One single fat cell bound up to 10000 insulin molecules and the effect on glucose oxidation was linearly proportional to the amount of insulin bound, an observation which supports assumption (2), used in the preceding section for the calculation of dose-response curves. The equilibrium (dissociation) constant of the insulin-receptor complex was 5.0×10^{-11} M. The controversy over hormone binding versus hormone destruction has recently been settled by FREYCHET (1972). He showed that liver-cell membranes actually did destroy insulin *in vitro* but that this destruction was not linked with the binding of the hormone to the receptor. The two processes, binding and destruction, were shown to be entirely independent. Analogous results were reported for the binding of ACTH to adrenal membranes (LEFKOWITZ, 1970) and for several other polypeptide hormones (see short review in CUATRECASAS, 1971b). RODBELL (1971a) reported that ^{125}I-labeled glucagon binds to isolated liver-plasma membranes. Again the binding was reversible and the biologic effect, namely the activation of the membrane-bound enzyme adenyl cyclase was

proportional to the amount of bound hormone. Some unexpected observations were made, however, in the glucagon-liver membrane system. Firstly, under certain conditions, the response of the target (the membrane-bound adenyl cyclase) is not only a function of the surrounding hormone concentration. Rather, nucleotides, in particular guanosine triphosphate (GTP), greatly influence the response to a given hormone concentration (RODBELL, 1971 b, 1971 c). Secondly, the binding of labeled hormone is not entirely reversible even by a 1000-fold excess of unlabeled hormone and the dissociation of the hormone-receptor complex does not obey simple first-order kinetics (RODBELL, 1971 a). Both findings are contrary to conventional theory about action of hormones.

Interesting compounds have been studied which specifically inhibit the response of the target organ to a number of hormones. Kinetic analysis has shown that in many cases the inhibition is of the *competitive* type. In physicochemical terms this means that the inhibitor binds to the hormone receptor, thereby blocking attachment of the hormone. Competitive antagonists usually show some chemical similarity to the corresponding hormone. Sometimes, however, competitive antagonists may be structurally quite different, which has led ARIËNS (1966) to conclude that they do not always interact with the same chemical groups of the receptors as the corresponding hormones but attach themselves to some neighbouring groups of the receptor at so-called accessory binding sites with part of their molecule extending into the region where the hormone should be bound. Thus, steric hindrance will prevent attachment of the hormone molecule. Spironolactone, which displaces aldosterone from its receptors, is a typical example of a competitive hormone antagonist (SHARP, 1966). Clomiphene and ethamoxytriphetol are hormones antagonistic to estrogens (LERNER, 1964), and cyproteron is an antagonist to androgens (NEUMANN, 1967). A glucagon derivative lacking the amino-terminal histidine has been found to act as a specific glucagon antagonist (RODBELL, 1971 d) and an ACTH-analogue may act as an ACTH antagonist (SEELIG, 1971). The antagonists mentioned so far are pharmacological substances which do not occur in nature. Naturally-occurring antagonists to insulin have been found in plasma by various authors who claim they are the cause of diabetes mellitus, but the significance of these substances is still debated. For completeness we must also mention here that immunological antibodies can arise to peptide hormones given by injection. Such antibodies can, for example, neutralize the action of insulin and may very well explain the insulin resistance found in some diabetics.

The chemical nature of receptors has been studied mainly by indirect means. RODBELL (1971 a, 1971 c) found that trypsin, phospholipase A and digitonin destroyed the glucagon-binding sites of liver membranes. He concluded that glucagon receptors were lipoproteins and the lipid and the protein moieties were both essential for proper receptor function. By contrast, insulin receptors in fat and liver cells seem resistant to the action of phospholipase, which suggests that they are chemically different compounds (CUATRECASAS, 1971 a). The receptors for both glucagon and insulin are firmly bound to particulate cell matter, most probably to the cell membrane. This has complicated the study of their chemical nature, but recently CUATRECASAS (1972) has succeeded in rendering insulin receptors of fat and liver cells soluble simply by treating the membranes with an non-ionic detergent. The soluble receptor turned out to be a protein, as had been predicted on the basis of the more indirect studies. Lipids are probably not an essential part in the function of this receptor. The estimated molecular weight was 300000.

Proteins seem particularly suited to the task of receptors since they are capable of binding a great variety of substances ranging from simple ions to large macromolecules. This property of proteins was extensively analyzed by SCATCHARD as early as 1949. MONOD (1966) discussed the possibility that hormones might be allosteric ligands, and hormone receptors allosteric proteins. Allosteric proteins are composed of two or more peptide chains (subunits) interconnected noncovalently by an incomplete fit. This incomplete fit gives rise to strain or tension in the folding of the protein chains. If a small molecular ligand now binds to a specific site of such a molecule, the strain will change, e.g. by a different electron or charge distribution, and the molecule will assume a slightly different shape (conformation). This change of shape is called an allosteric transition. Since the transition involves the whole protein molecule, sites quite distant from the original ligand-binding site may be affected, e.g. the catalytic site of an enzyme (MONOD, 1965). The concept that receptors are allosteric proteins is appealing. Thus, allosteric proteins can receive signals, transmit them along the protein-chain and translate them into another signal. According to SCHWARTZ (1966) these are exactly the properties which are required of receptors. Furthermore, allosteric proteins are also capable of greatly amplifying signals (MONOD, 1965) – they act, so to speak, as molecular amplifiers

and relay stations. BOWNESS (1966) and GRAHAME-SMITH *et al.* (1967) were actually able to demonstrate that adenyl cyclase, a possible component of the receptor for several hormones, functions as an amplifier for hormonal signals. In spite of this evidence MONOD was reluctant to support the view that hormone receptors, especially in the case of steroid hormones, are allosteric proteins. Our knowledge of the chemical nature of receptors is still so inadequate that the question must remain unsettled, but very recently BAXTER (1972) has provided good evidence that the so-called cytoplasmic receptors for glucocorticoid hormones are in fact allosteric proteins.

H. Events Following the Binding of Hormones to Receptors

Well-defined biochemical effects for practically every hormone have been described and quantitatively measured in a great variety of *in-vivo* and *in-vitro* test systems using the corresponding target organ. These well-known hormone effects are, however, not necessarily the direct consequence of the first step of hormone action, the attachment to the receptor. Thus it is not known, for example, which of the physicochemical events ultimately leading to acceleration of glucose transport occur after binding of insulin to the fat-cell membrane. The almost complete chain of events linking binding at the receptor site to the biochemically evident hormone effect has so far only been established for the action of glucagon and epinephrine on the breakdown of liver glycogen (see cyclic AMP, below). For most other hormones, virtually nothing is known of the reaction after the receptor site has been occupied, a step that has sometimes been referred to as the *primary action* of a hormone. In view of the facts now available on the glucagon-adenyl cyclase-cyclic AMP system this term is hardly appropriate, since this "primary action" may in fact be a cascade of several chemical events. Despite the gaps in our understanding of the precise chemical actions of hormones a classification according to their effects on cellular processes will be presented below. Such a classification will of course remain tentative and will have to be modified as more facts emerge.

1. Effects of Hormones on Membranes

Classically, membrane effects imply actions of hormones upon the permeability of membranes (usually the cell membrane) to small molecules or upon transport mechanisms within such membranes. Certain hormones which activate enzymes bound to the cell membrane (e.g. adenyl cyclase) without necessarily influencing permeability or transport mechanisms may also be included at this point. Insulin can be considered as the prototype of a hormone which acts on membrane properties. As was shown by LEVINE (1955), the main action of this hormone is to increase the transport of glucose from the extracellular into the intracellular fluid. This effect has been demonstrated in both muscle and adipose tissue (PARK, 1959; CROFFORD, 1965). In both tissues, glucose is transported through the cell membrane by means of a carrier (not yet identified) which is thought to bind glucose on one side of the membrane and release it into the cell on the other side. The following properties are characteristic of this type of carrier transport: 1. There is strict stereochemical specificity of the carrier to the substrate. For example, D-glucose is transported via the carrier mechanism whereas L-glucose is not. 2. Carrier transport obeys saturation kinetics and by this means differs from transport via simple diffusion. 3. Carrier transport does not require energy (so-called "facilitated diffusion") unless the substrate is transported against an energy or concentration gradient, in which case the carrier transport must be coupled to an energy-producing process. In the latter case the term "pump mechanism" has been applied. 4. Under suitable experimental conditions, counter-transport may be observed, i.e. the substrate is transported against a concentration gradient but not against an energy gradient. In erythrocytes, muscle and adipose tissue, glucose transport fulfills these criteria (WILBRANDT, 1961; PARKS, 1959; CROFFORD, 1965). In the last two tissues insulin appears to produce an increase in "carrier places" or in the affinity of the carrier for glucose (CROFFORD, 1965). For the sake of completeness it must be mentioned here that insulin has other effects which seem to be independent of glucose transport. It affects uptake of amino acids, lipolysis and protein synthesis in several tissues.

Aldosterone increases the transport of sodium ions from the tubular lumen across the renal tubular cells. SHARP (1966), using the toad bladder as an experimental model for the renal tubule, showed that aldosterone promoted the transport of sodium ions through the apical cell membrane. This effect appears only with a latent period of 45–120 min after the hormone has been added. Aldosterone has no effect if protein synthesis is blocked with puromycin or if energy production is inhibited in the toad bladder by the omission of substrate (glucose

or pyruvate). The transport of sodium ions which is induced by alosterone is thus coupled with an energy-providing process, not an unexpected finding since sodium ions are transported against a concentration gradient.

2. Cyclic AMP and the Concept of the Second Messenger

Parathormone and vasopressin are two other hormones which act upon transport mechanisms within the kidney. The former inhibits phosphate transport and the latter increases water permeability across the cells of the renal tubules. It is likely that both hormones exert their effect by increasing the conversion of adenosine triphosphate (ATP) to 3′,5′-adenylic acid (cyclic AMP), a reaction catalyzed by the membrane-bound enzyme adenyl cyclase. This fundamental mode of hormone action was discovered by SUTHERLAND in 1958 during studies on the glycogenolysis induced by glucagon and epinephrine in liver tissue. At that time it was known that glucose-1-phosphate was split off from glycogen by an enzyme, phosphorylase, and that this enzyme existed in the inactive b form and the active a form. SUTHERLAND stimulated glycogenolysis by incubating liver tissue in a medium containing glucagon. From homogenates of this stimulated tissue he recovered a heat-stable substance which was capable of activating phosphorylase in homogenates of untreated liver tissue. This small molecular substance was isolated and rapidly identified as 3′,5′-adenylic acid (cyclic adenosine monophosphate, cyclic AMP). Fig. 7 shows the structural formula of this interesting nucleotide.

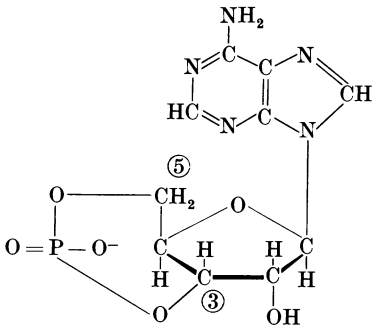


Fig. 7. Formula of 3′,5′-adenylic acid (cyclic AMP)

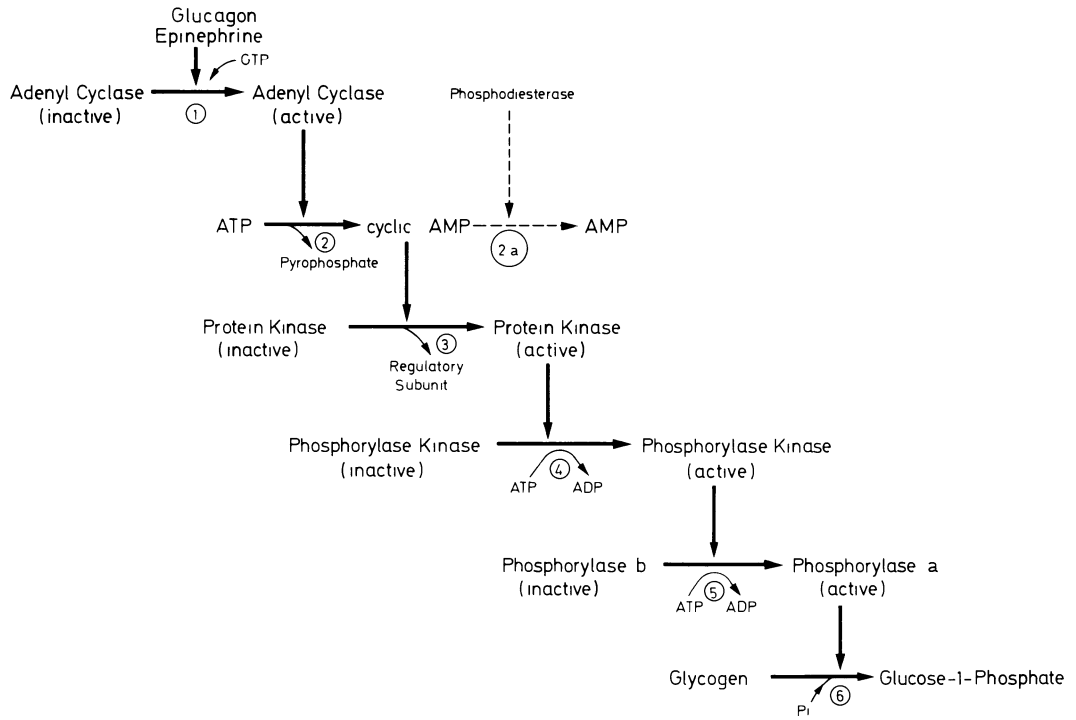


Fig. 8. Cascade of events leading to the activation of glycogen breakdown by glucagon or epinephrine in the liver. Most steps can now be exactly defined in physicochemical terms. 1. Binding of hormone to the receptor and activation of adenyl cyclase. This process is still poorly understood. Guanosine triphosphate (GTP) interferes with this process in a complex manner. 2. Formation of cyclic AMP from ATP. 2a. Enzymatic degradation of cyclic AMP. 3. Cyclic AMP binds to a regulatory subunit of the protein kinase. This leads to dissociation of the regulatory subunit from the catalytic subunit, the latter becoming active. 4. Phosphorylation of the inactive phosphorylase kinase. 5. Phosphorylation of the inactive phosphorylase. 6. Removal of glucose-1-phosphate from glycogen by phosphorolysis. Abbreviations: ATP: Adenosine triphosphate; ADP (AMP): Adenosine-di-(mono) phosphate; GTP: Guanosine triphosphate; Pi: Inorganic phosphate

Cyclic AMP is produced from adenosine triphosphate (ATP) through the enzyme adenyl cyclase. Cyclic AMP in turn is degraded into 5'-AMP by a specific enzyme termed phosphodiesterase. Phosphodiesterase is inhibited by methyl xanthines, such as caffeine and theophylline, a phenomenon which may explain some of the pharmacological effects of these substances. The exact mechanism by which cyclic AMP finally leads to the activation of the enzyme phosphorylase in muscle has been unravelled quite recently. The first step consists in the binding of cyclic AMP to a regulatory subunit of an inactive enzyme, protein kinase. This causes the dissociation of the regulatory from the catalytic subunit of this enzyme, which thereby becomes active (GILL, 1971). The active protein kinase phosphorylates (and thereby activates) the phosphorylase kinase which in turn activates phosphorylase (WALSH, 1971). The complete sequence of events is shown in Fig. 8, which shows that nearly every chemical step, from the attachment of the hormone at the receptor to the final hormonal effect, glycogenolysis, can be exactly defined. The only remaining uncertainty is how the binding of the hormone will activate adenyl cyclase, a question that seems particularly difficult to solve because both receptors and adenyl cyclase are firmly bound to the cell membrane and have not been obtained in soluble form yet. As will be discussed below, some authors have postulated that adenyl cyclase and receptor are identical.

In the few years since cyclic AMP was discovered in the liver this nucleotide has also been identified in many other tissues. The concentration of cyclic AMP in other tissues is also under hormonal control. The list of hormones whose actions may be transmitted through cyclic AMP is now long (Table 1) and several extensive reviews dealing with this subject have already been published (SUTHERLAND, 1966; ROBISON, 1968). Cyclic AMP appears to transmit the actions of so many hormones that SUTHERLAND has called it a "second messenger". According to SUTHERLAND'S concept, the hormone in extracellular fluid represents the "first messenger". The contact between hormone and adenyl cyclase leads to activation of the latter with the formation of cyclic AMP which transmits the hormone effects intracellularly by virtue of its activity as the second messenger. Fig. 9 is a schematic representation of the function of the second messenger. In this diagram adenyl cyclase is identical to the hormone receptor, an interpretation which may be an oversimplification, but has received the following indirect experimental support: 1. β-adrenergic receptor-blocking agents inhibit the catecholamine activation of adenyl cyclase in different tissues. The similarity between adenyl cyclase and β-receptors is so striking that ROBISON (1967) has postulated that β-receptors are identical to adenyl cyclase. 2. All hormones whose effects are based on an increase of cyclic AMP cause adenyl cyclase to be converted from an inactive into an active form. The *de-novo* synthesis of adenyl cyclase under the influence of hormones (which would be incompatible with a receptor function of this enzyme) has been excluded experimentally. Thyroxine may represent a possible exception to this statement, since in adipose tissue it has been reported to induce *de-novo synthesis* of adenyl cyclase (KRISHNA, 1968), whereas in myocardial tissue it appears to act like other hormones by causing the *activation* of the enzyme (LEVEY, 1969). 3. Adenyl cyclase appears to be firmly bound to membrane structures, probably the cell membrane, in all tissues investigated so far. In fact, an active hormone-sensitive enzyme has not yet been obtained in soluble form.

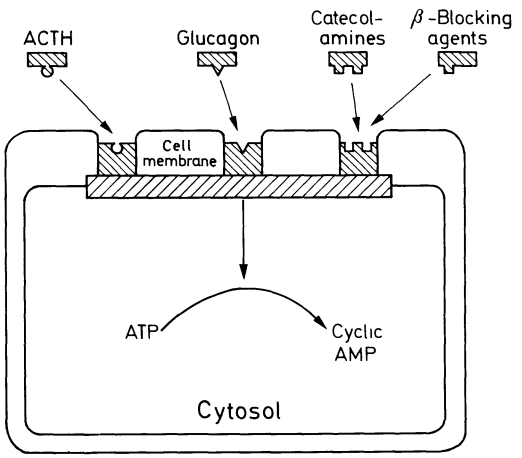


Fig. 9. Adipose-tissue cell with three different regulatory subunits that serve as hormone-specific receptors. There is a single catalytic subunit pointing toward the interior of the cell. Hormones or antagonists bind to the regulatory subunits. This simplified representation is based largely on experiments of BIRNBAUMER (1969). The exact mode whereby the binding of the hormone activates the catalytic subunit is still poorly understood.

One of the fundamentals of endocrine control is that each hormone exerts a specific action on a particular tissue. The numerous hormones listed in Table 1 which activate adenyl cyclase, and the large number of actions which are initiated by cyclic AMP appear to contradict this concept of hormone specificity. In other

Table 1. Survey of hormones whose action presumably involves 3′,5′-adenylic acid (cyclic adenosine monophosphate) as "second messenger"

Hormone	Organ or tissue	Level of cyclic AMP	Enzymes or chemical steps, influenced by cyclic AMP		End effect	
Catecholamines	Liver	+	Glycogen-Synthetase	−	Glycogen synthesis	−
			Phosphorylase	+	Glycogenolysis	+
			Pyruvate → Phosphoenolpyruvate	+	Gluconeogenesis	+
	Adipose tissue	+	Triglyceride-Lipase	+	Lipolysis	+
	Myocardium	+	Phosphorylase	+	Glycogenolysis	+
			unknown		Inotropism	+
	Skeletal muscle	+	Phosphorylase	+	Glycogenolysis	+
Glucagon	Liver	+	As for catecholamines		As for catecholamines	
	Adipose tissue	+				
	Myocardium	+				
	Pancreas (β-cell)	(+)	Unknown		Insulin-Release	
Insulin	Adipose tissue[a]	−	Triglyceride lipase	−	Lipolysis	−
	Skeletal muscle	[b]	Phosphorylase	−	Glycogenolysis	−
			Glycogen synthetase	+	Glycogen synthesis	+
	Liver[a]	−	Glycogen synthetase	+	Glycogen synthesis	+
			Phosphorylase	−	Glycogenolysis	−
			Pyruvate → Phosphoenolpyruvate	−	Gluconeogenesis	−
ACTH	Adrenal cortex	+	Cholesterol → Pregnenolone	+	Glucocorticoid synthesis	+
	Adipose tissue	+	Triglyceride lipase	+	Lipolysis	+
TSH	Thyroid gland	+	Phosphorylase	+	Glycogenolysis	+
			Unknown		Uptake and organification of iodine, hormone release	+
	Adipose tissue	+	Triglyceride lipase	+	Lipolysis	+
LH (ICSH)	Corpus luteum	+	Cholesterol → Pregnenolone	+	Progesterone synthesis	+
	Testis	(+)	Cholesterol → Pregnenolone	+	Testosterone synthesis	+
MSH	Frog skin	+	Unknown		Melanophore dispersion	+
Vasopressin	Kidney	+	Unknown		Tubular water reabsorption	+
Parathormone	Kidney	+	Unknown		Tubular reabsorption of phosphate	−
	Bone	+	Unknown		Resorption of bone	+
Thyroxine	Adipose tissue	+[c]	Neoformation of Adenyl cyclase		Sensitivity to catecholamines	
	Myocardium	+	As for catecholamines		As for catecholamines	

Key: + increase; − decrease; (?) probable increase, but not proven directly; ? unknown type of action.
[a] Action of insulin on adipose tissue and liver is only definitely measurable when adenyl cyclase is previously activated, e.g. by glucagon.
[b] Action of insulin on the level of cyclic AMP in skeletal muscle is controversial.
[c] In contrast to all other hormones, thyroxine does not seem to lead to activation of adenyl cyclase but rather to a neoformation of this enzyme (see text) in adipose tissue.
References can be found in the reviews by E. W. SUTHERLAND (1966) and G. A. ROBISON (1968).

words, if all tissues were to possess an identical adenyl cyclase then catecholamines, glucagon, ACTH and several other hormones would have exactly the same effects upon them. This, of course, is not so. ROBISON and co-workers (1967) have therefore suggested that adenyl cyclase consists of at least two subunits, one being regulatory and varying from tissue to tissue and the other being catalytic and possibly identical in all tissues. The regulatory subunit would function as the true receptor which binds only with the specific hormone, thereby activating the catalytic subunit. This interpretation is supported by several different experimental results. For example, ACTH, glucagon and catecholamines promote lipolysis in adipose tissue through the activation of adenyl cyclase. The action of catecholamines, but not that of ACTH and glucagon can be inhibited through β-receptor blocking agents. This indicates at least two different regulatory subunits (receptors) of adenyl cyclase. Furthermore, data published by BALLY (1968) suggest that there are probably two separate receptors for ACTH and glucagon. According to this concept,adipose tissue adenyl cyclase would therefore have a specific receptor for each of the three hormones considered, as is shown in Fig. 9.

Cyclic AMP has not been accepted without reservations as the almost universal second messenger of hormones and some questions remain unsolved. For example, it is disturbing that the concentration of cyclic AMP is many times higher even without hormonal stimulation in adipose tissue and in the liver than is necessary for full activation of the triglyceride lipase and phosphorylase in tissue homogenates. In order to explain this discrepancy, it has been postulated that the major part of cyclic AMP is compartmentalized within the cell segregated from the protein kinase. This speculation awaits experimental verification. Another still unexplained observation has been made in studies on the effect of insulin on glycogen synthetase of muscle. As in liver and adipose tissue insulin stimulates this enzyme; however, in muscle insulin produces a rise in cyclic AMP instead of the expected fall (GOLDBERG, 1967). Nevertheless, despite the objections that may be raised, the concept of the second messenger must be considered an important contribution to our understanding of the action of hormones.

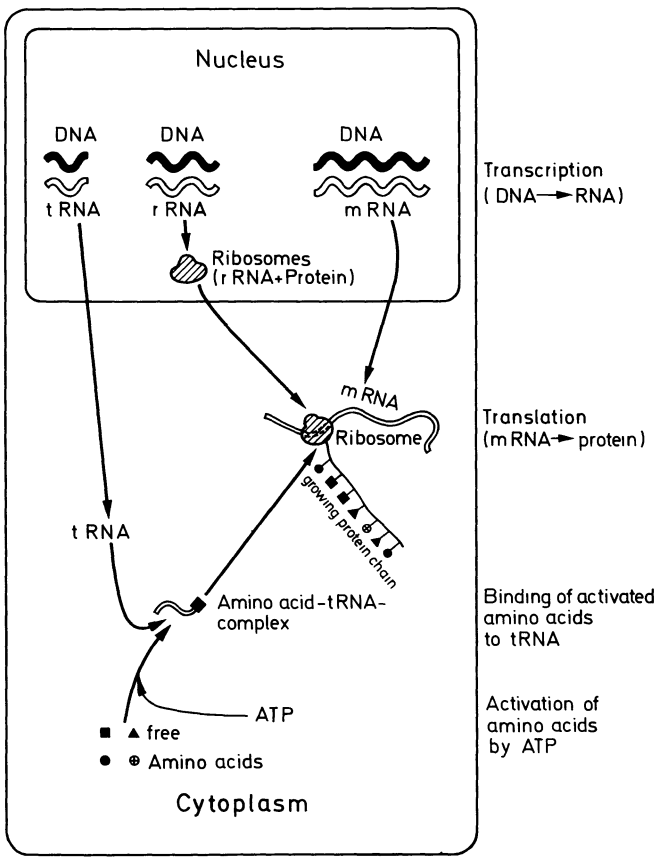


Fig. 10. Schematic representation of protein synthesis in a cell. Abbreviations: DNA: deoxyribonucleic acid; tRNA: transfer ribonucleic acid; rRNA: ribosomal ribonucleic acids; mRNA: messenger ribonucleic acid; ATP: adenosine triphosphate

3. Effects of Hormones on Protein Synthesis

CLEVER (1960) described that ecdyson, an insect hormone, led to swelling, so called "puff" formation, at specific locations on the giant chromosomes of the midge Chironomus tentans. In the larvae of these insects ecdyson induces enzymes that alter cuticular proteins and produce pupation. Since it was already known from other studies that chromosome "puffs" corresponded to active genes, CLEVER came to the exciting conclusion that ecdyson exerted its effect directly on the cell nucleus, where it activated a specific gene and thereby caused the induction of an enzyme protein. Since this discovery, the actions of many hormones on protein synthesis have been intensively investigated, an area of research that TATA (1968) and KORNER (1970) have briefly reviewed. For the understanding of the actions of hormones upon protein synthesis we shall first summarize the biochemical steps needed for building proteins (Fig. 10). Most of the knowledge in this field has been gained within the last 20 years from work on bacteria. However, the results can also be applied to mammalian cells with few modifications. We shall limit the discussion to those steps in protein synthesis which are essential for the understanding of the actions of hormones. For more detailed descriptions the reader is referred to standard biochemistry textbooks. The information for the production of all proteins required is stored in the form of genes within chromosomes in the nucleus of every cell. Chemically, genes are long strands of deoxyribonucleic acids (DNA) which contain the information for protein synthesis in the form of a variable sequence of four bases, adenine, thymine, cystosine and guanine. If a particular protein is to be produced, a negative copy with a complementary base sequence is first obtained from the DNA of the corresponding gene in the form of a strand of ribonucleic acid (messenger RNA = mRNA). This process is called transcription. mRNA migrates from the nucleus into the cytoplasm and comes into contact with the ribosomes. The ribosomes are small particles which move along the strands of mRNA and read the sequence of bases and put together the correct amino acids to form a peptide chain. In this process, the base sequence is translated into an amino-acid sequence (translation) according to the specific genetic code. Ribosomes themselves consist partly of ribonucleic acids, so-called rRNA. A specific transport RNA (tRNA), a third type of ribonucleic acid, combines with each of the 20 individual amino acids in the cytoplasm and facilitates their contact with and recognition by the ribosomes. rRNA and tRNA are both produced in the nucleus through transcription from DNA. It is easy to imagine from Fig. 10

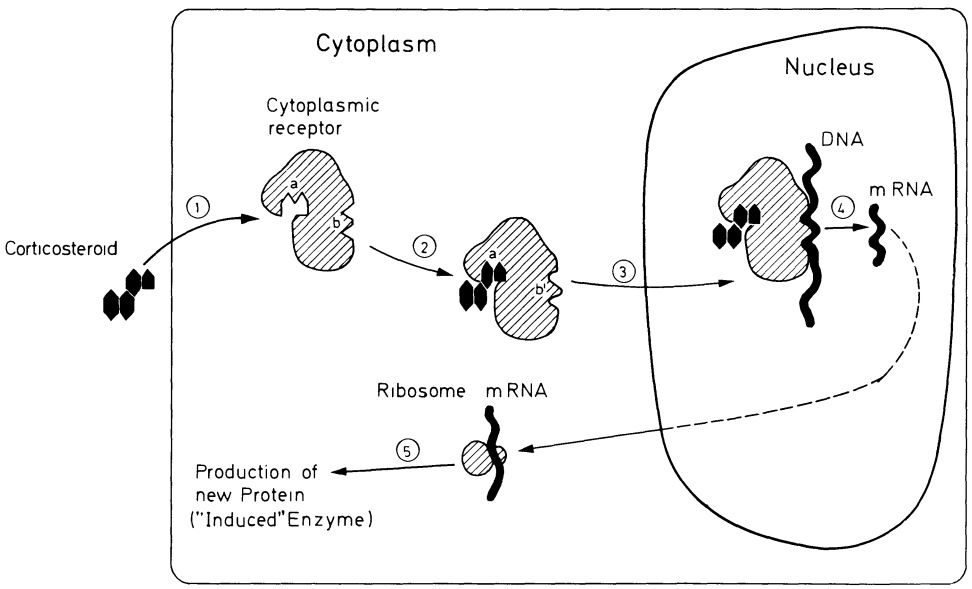


Fig. 11. Schematic representation of the mode of action of corticosteroid hormones. The stages are: 1. The steroid hormone penetrates into the cell. 2. In the cell cytoplasm it binds reversibly to the binding site (a) of a soluble receptor protein. Thereby it induces a slight change in shape of this molecule, notably in the region (b). 3. The hormone-receptor complex travels to the nucleus, and thanks to the slight alteration the region (b) fits on to nuclear DNA. 4. The binding of the complex to DNA causes the transcription of messenger RNA. 5. This ultimately leads to the production of a new protein, e.g. the enzyme tyrosine aminotransferase, on the ribosomes. This simplified representation is based on results by BAXTER *et al.* (1972). Similar mechanisms of action have been described for many other steroid hormones

that hormones may theoretically control different steps of protein synthesis, e.g. transcription from DNA into mRNA, rRNA and tRNA, or translation of mRNA into protein, not to mention the uptake of amino acids from the extracellular fluid.

So far, effects on protein synthesis have only been seen when intact cells (in vivo or in vitro) have been exposed to a hormone. Cell-free protein-synthesizing systems do not seem to be hormone-sensitive. For this reason there has been difficulty in identifying the exact mechanism of hormonal effects on protein synthesis. Nevertheless, experiments performed with chemical inhibitors of specific steps of protein biosynthesis have provided indirect evidence of the site of action. The most important conclusion to be drawn from the available data is that the mode and site of action may vary from one hormone to another, as will be seen from the following discussion and the summary in Table 2.

KORNER (1965) noted that growth hormone injections into rats produced an increase in protein synthesis in the liver. The hormone effect was demonstrable in cell-free liver homogenates, provided the hormone had been injected into the rats *in vivo*. Comparison of the activity of ribosomes from treated and untreated animals suggested that growth hormone primarily increased the concentration of mRNA in the cytoplasm. Since actinomycin D (an antibiotic which inhibits transcription of DNA in the nucleus) prevented the action of growth hormone, KORNER concluded that growth hormone promoted mRNA synthesis, i.e. transcription. It is probable that growth hormone also causes increased synthesis of rRNA. HAMILTON (1968) studied the action of a single injection of estradiol on protein synthesis in the uterus of ovarectomized rats. The results are summarized in Fig. 12. It can be seen that estradiol produces maximal stimulation of RNA synthesis in the nucleus within a few minutes. An increase in ribosomes and newly formed proteins could only be demonstrated after several hours. Analogous effects with slightly different timing are produced by testosterone on the prostate and seminal vesicles, and by corticosteroids and triiodothyronine on the liver (see TATA'S review, 1968). Matters are rather more complicated in the case of triiodothyronine than in that of the other hormones mentioned, since triiodothyronine causes stimulation of protein synthesis very rapidly, before RNA synthesis is increased. This effect is dependent upon the presence of mitochondria, for if mitochondria are absent *in vitro*, this early effect is not observed (SOKOLOF, 1968). The early triiodothyronine effect is probably related to the stimulation of mitochondrial respiration. Only in a later phase does triiodothyronine stimulate mRNA and ribosomal protein synthesis independently of mitochondria.

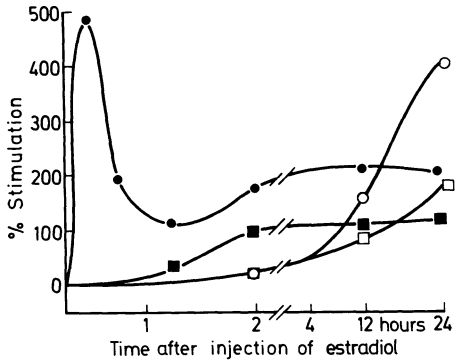


Fig. 12. Effect of a single injection of estradiol on protein synthesis in the uterus of ovarectomized rats. Tritium-labeled uridine was injected at the same time as estradiol ●–● specific activity of RNA in the nucleus; ■–■ activity of RNA polymerase; ○–○ ribosome concentration in the cytoplasm; □–□ total tissue protein (per mg DNA). Abbreviations as in Fig. 10. (Simplified from T. H. HAMILTON, 1968)

In summary, growth hormone, estradiol, testosterone, glucocorticoids and thyroid hormone (with the exception of the "early" effect of the last) can be said to control protein synthesis at the stage of *transcription*. This interpretation also appears to be valid when the hormone does not stimulate but rather inhibits protein synthesis as in the action of cortisol in lymphocytes (MAKMANN, 1967).

Insulin enhances protein synthesis in muscle. This effect is independent of the well-known stimulation of glucose transport across the cell membrane. Insulin appears to increase RNA synthesis by stimulating transcription. However, it can also stimulate protein synthesis even when RNA synthesis is fully blocked with antibiotics. WOOL (1966) therefore concluded that insulin may also enhance protein synthesis at the ribosomal stage (translation). Subsequent experiments by A. G. SCHWARTZ and AMOS (1968) have confirmed the action of insulin on ribosomes and have demonstrated that ribosomes from fibroblasts cultured in a medium containing insulin synthesize more protein than ribosomes from fibroblasts cultured without insulin. In cell cultures of liver tumors cortisol does not stimulate global protein synthesis. Rather it causes a specific enzyme (tyrosine transaminase) to be synthesized. THOMPSON'S (1966) experiments have suggested that in this instance cortisol acts at the stage of translation rather than that of transcription. However, in

a different liver tumor-cell line the glucocorticoid effect has recently been located to transcription again (BARNETT, 1971). Moreover, cyclic AMP has been found to have a permissive effect on the enzyme induction (SAHIB, 1971).

Other hormonal effects on protein synthesis are summarized in Table 2. In general, the available evidence points to the fact that endocrine control of protein synthesis is not exerted at one metabolic step identical for all hormones. The site of action seems to vary from hormone to hormone and from tissue to tissue. Several hormones, e.g. insulin and cortisol, appear to act at several steps of protein synthesis. It has been said that all gonadal and adrenal steroid hormones act on the transcription of DNA into RNA in the cell nucleus. Table 2 confirms this impression, but it is wise to remember KARLSON's warning (1961) against simplifying generalizations in this field.

The exact molecular or chemical mode of action of hormones on the cell nucleus and on ribosomes remains unsolved. The opinion has been expressed that hormone receptors in the cell nucleus are identical with so-called gene *repressors*. These are proteins which combine reversibly with DNA strands and prevent transcription (BRETSCHER, 1968). Repressors can combine with derepressors at a second binding site. Attachment of the derepressor slightly distorts the shape of the repressor molecule which thereby loses its affinity for DNA. The repressor then dissociates from DNA which is cleared for transcription. Recent evidence suggests that a sequence of events roughly resembling the one postulated in this hypothesis actually takes place in the case of steroid hormones. The mechanism suggested by BAXTER (1972) has been outlined in more detail on p. 8 and in Fig. 11.

Many of the biochemical observations discussed above may be disappointing to both clinicians and physiologists, partly because they deviate widely from known clinical hormone actions and partly because they appear to contradict the concept of specificity of hormone action. For example, the observation that cortisol increases global protein synthesis instead of exerting its expected catabolic effect upon the liver is disturbing. On the other hand, it must be appreciated that the results of biochemical experiments are reasonably consistent with clinical and physiological observations in the case of the effects of testosterone on the prostate, estradiol on the uterus, and possibly growth hormone upon the liver, and agreement is excellent in the effects of cortisol on hepatoma cells in tissue culture and of prolactin on mammary tissue in organ culture. In both cases the synthesis of a single specific enzymic protein is induced without stimulation of global protein synthesis, and the enzyme induced explains some of the clinical effects of the hormone (Table 2).

4. Direct Effects of Hormones on Enzyme Activities

We shall discuss here the ability of hormones to activate or inactivate enzymes without the formation of new enzyme protein. It is important to appreciate that this process is separate from the induction of enzyme synthesis (e.g. of tyrosine transaminase under the influence of cortisol which necessitates synthesis of new enzyme protein and which has been discussed in the previous section).

A classic example of an enzyme whose activity can be increased or inhibited by different hormones is that of adenyl cyclase (see section on cyclic AMP). Despite concerted efforts to reveal other enzymes which serve as hormone receptors, few analogous hormone-enzyme interactions have been described to which one can attribute physiological significance. The earlier hypothesis that the action of insulin on glucose metabolism may be due to activation of hexokinase is no longer generally accepted. TOMKINS (1963) has surveyed the action of steroids on isolated enzymes and has distinguished two mechanisms of interaction. In one case the hormone acts as a *co-factor* on enzymatic reactions. The transhydrogenase function of estradiol may serve as an example. In the second case the steroid hormone can influence the activity of an enzyme by reversibly altering the enzyme's tertiary and quaternary structure. Glutamate dehydrogenase and pyruvate kinase are examples of two enzymes controlled by steroids in this way. Thyroxine leads to dissociation of glutamate dehydrogenase into subunits (WOLFF, 1962) and to inactivation of alcohol dehydrogenase (MCCARTHY, 1968), the latter probably through an allosteric effect. It is difficult to attribute physiological significance to these actions of steroid and thyroid hormones. The phenomena described above may well be test-tube effects unrelated to the physiological function of the hormones in the intact cells. The actions of thyroxine and triiodothyronine on the metabolism of mitochondria must be discussed in this context. In this case the thyroid hormones are assumed to influence oxygen consumption and oxidative phosphorylation in a complex multi-enzyme system involved in electron transport. For detailed discussion of these processes the reader is referred to reviews compiled by HOCH (1962) and TAPLEY (1967).

Table 2. Hormones which presumably act on protein synthesis, or which are dependent on protein synthesis for their action

Hormone	Organ or tissue	Effect of hormone on	Presumed site of action		References
			Transcription	Translation	
Cortisol	Liver	Incorporation of phosphate into tRNA in vivo	+		W. D. WICKS (1965)
	Liver	Incorporation of phosphate into rRNA in vivo	+		D. L. GREENMAN (1965)
	Liver tumor	Synthesis of tyrosine transaminase in cell cultures		+	E. B. THOMPSON (1965)
	Liver tumor	Synthesis of phosphoenolpyruvate carboxykinase and tyrosine transaminase in cell cultures	+		C. A. BARNETT (1971) M. K. SAHIB (1971)
	Lymphocytes	Incorporation of amino acids into microsomal protein in vivo and in vitro. Complex mode of action, partly involving transport into the cells of amino acids	−		A. PENA (1966) M. H. MAKMAN (1968)
Testosterone	Prostate	Activity of mRNA in nucleus and ribosomes after hormone injection in vivo	+		S. LIAO (1965)
	Seminal vesicle	Incorporation of phosphate into tRNA in vivo	+		W. D. WICKS (1965)
	Seminal vesicle	Incorporation of phosphate into rRNA in vivo	+		D. L. GREENMAN (1965)
Estradiol (and other estrogens)	Oviduct (chick)	Activity of RNA-polymerase, synthesis of mRNA coding for ovalbumin	+		B. W. O'MALLEY (1969, 1972)
	Uterus	Incorporation of uridine into nuclear DNA in vivo	+		T. H. HAMILTON (1965)
	Uterus	Synthesis of "induced protein"	+		B. S. KATZENELLENBOGEN (1972) E. E. BAULIEU (1972)
Progesterone	Oviduct (chick)	Activity of RNA-polymerase, synthesis of avidin	+		B. W. O'MALLEY (1969, 1972)
Aldosterone	Toad bladder	Hormone effect on Na^+ transport in vitro dependent upon RNA synthesis	+		I. S. EDELMAN (1963)
Insulin (The main action of this hormone is to promote glucose transport into cells)	Muscle	Incorporation of adenine into RNA in vitro	+		I. G. WOOL (1963)
	Muscle	Protein synthesis by ribosomes in a cell-free system (hormone administered in vivo)		+	I. G. WOOL (1966)
	Liver tumor	Synthesis of tyrosine transaminase in cell culture (presence of glucocorticoid required)		+	T. D. GELEHRTER (1970)
	Fibroblasts	Protein synthesis in a cell-free system by ribosomes derived from fibroblasts cultured in the presence of insulin	+?		A. G. SCHWARTZ (1968)
Growth hormone	Liver	Protein synthesis in a cell-free system (hormone administered in vivo)	+		A. KORNER (1965)
Prolactin	Mammary gland	Synthesis of lactose synthetase in organ culture	+		R. W. TURKINGTON (1968a)
	Mammary gland	Synthesis of casein in organ culture	+		R. W. TURKINGTON (1968b)

Table 2 (continued)

Hormone	Organ or tissue	Effect of hormone on	Presumed site of action		References
			Transcription	Translation	
Thyrotropic hormone	Thyroid gland	Incorporation of adenine into RNA by isolated cell nuclei in vitro	+		D. J. BEGG (1965)
	Thyroid gland	Incorporation of adenine into RNA by tissue slices in vitro. Effect possibly secondary to an enhancement of carbohydrate metabolism	+		R. HALL (1968)
Triiodothyronine	Liver	Incorporation of uridine into nuclear RNA and activity of RNA polymerase in vivo	+		J. R. TATA (1968)
	Liver	Protein synthesis in a cell-free system (hormone administered in vivo). Effect partly dependent on the presence of mitochondria.		+	L. SOKOLOFF (1968)

Key: + stimulation; − inhibition. There is no claim that the table is complete.

5. Effects of Hormones on Cellular Differentiation

It has long been known that certain hormones exert impressive effects on the differentiation of cells and organs. The study of organ differentiation has moved a long way in the past ten years from the classic morphologic description of embryogenesis to a biochemical understanding of the processes involved. The differentiation of a cell depends ultimately on the type and amount of enzymes it contains. The enzymes and structural proteins present in a cell will determine largely what metabolic functions the cell can perform, what substances it will take up, metabolize, store or secrete, and also what shape the cell will assume. Every nucleated cell of a given organism contains the identical full set of genes and is theoretically capable of synthesizing all the enzymes or proteins that any other cell in the same body produces. Cell differentiation therefore depends entirely on the fact that only some of the possible proteins are synthesized, while the expression of other genes is inhibited. It was therefore a safe prediction that differentiation could only be controlled by modulation of the expression of genes.

This prediction has now been largely confirmed by experiments in two interesting model systems, differentiation of the oviduct of immature chicks and metamorphosis of anurae. The former system has been extensively studied by O'MALLEY (1969). He found that the oviduct of immature chicks, which is lined with a flat undifferentiated epithelium, rapidly differentiates and starts to produce the protein ovalbumin when estrogenic hormones are injected. When progesterone is given after several days of estrogen treatment another very specific protein, avidin, is synthesized. The following facts, among others, suggest that hormones exert their control at the transcription stage (see Fig. 10): 1. The enzyme DNA-dependent RNA polymerase, which is necessary for transcription, is present in much larger quantities in the nuclei of estrogen-treated animals than in those of nontreated animals. 2. Estrogen treatment leads to the synthesis of large amounts of novel RNA which is not present in the untreated oviduct (probably mRNA). 3. Actinomycin D, a drug which selectively inhibits the process of transcription, abolishes hormonal effects on differentiation. The fact that estrogens also cause an increase in the amount of available tRNA (see Fig. 10) points to an additional control of differentiation at the stage of translation.

Biochemical studies on anuran metamorphosis have shown that the morphological changes are accompanied by numerous changes in the enzyme patterns of many organs (FRIEDEN, 1967). COHEN (1970) and GRISWOLD (1972) have focused their attention on the appearance of enzymes necessary for the production of urea. These enzymes are lacking in the tadpole, which excretes ammonia, but are present in the frog, which excretes urea. The level of some of these enzymes, e.g. carbamyl phosphate synthetase, increases 30-fold during metamor-

phosis. Injection of thyroxine greatly accelerates this change of enzyme. It has been clearly demonstrated that thyroxine does not produce *activation* of an enzyme protein already present but rather *induces* the production of new enzyme protein. As in the case of estrogen action in the chick oviduct, thyroxine produced a manifold rise in the enzyme DNA-dependent RNA polymerase. Thus the hormone affected transcription, but again additional points of control were identified at the stage of translation (COHEN, 1970).

In summary, all available data suggest that hormonal effects on differentiation must be considered in the more general context of the hormonal control of protein synthesis, which has been dealt with in the previous section. Many questions still remain unsolved in this field. For example the attentive reader will immediately point out that in the system mentioned above the chick oviduct must have undergone some differentiation before it was exposed to estrogen, since it appears to be one of the few tissues that responds to estrogens, while many other primitive epithelia, e.g. in the gut, do not change upon estrogen treatment.

I. Hormones as Components of Physiological Control Systems*

The previous sections have shown that endocrine systems are involved in the transfer of information by means of chemical signals with the ultimate goal of controlling biological processes. It is therefore possible to analyze endocrine processes with the aid of the *theory of control systems*.

In order to get acquainted with a simple control system we shall first analyze the problem of blood-sugar regulation in eviscerated rats. These rats lack both the hormones necessary for the control of the blood sugar (insulin, glucagon, adrenaline) and the organs supplying glucose (liver, kidneys, intestines). Since various tissues are in constant need of glucose, we must keep the rats alive by supplying them with external glucose in the form of an infusion. This is presented schematically in Fig. 13. We first select an infusion rate i which is equal to the consumption rate of glucose c, and the blood sugar level will remain constant. If we now double the infusion rate the blood sugar will rise. If the consumption rate c remained constant, the blood sugar would slowly rise to an infinite value. In actual fact however, after a certain

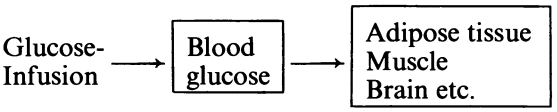


Fig. 13. Schematic representation of blood sugar control in an eviscerated rat infused with glucose

time the blood sugar adjusts itself to a new constant level (SOSKIN, 1937), because the rise in the blood-sugar level accelerates the reversible reaction

$$\begin{matrix}\text{Glucose} \\ + \\ \text{membrane} \\ \text{carrier}\end{matrix} \rightleftarrows \begin{matrix}\text{Glucose-} \\ \text{carrier} \\ \text{complex}\end{matrix} \rightarrow \begin{matrix}\text{intra-} \\ \text{cellular} \\ \text{glucose}\end{matrix}$$

from left to right, causing more glucose to be removed from the blood by the tissues. This simple control mechanism is due entirely to the law of mass action and is therefore named *passive control.* It can assure only incomplete homeostasis. To compensate for gross disturbances in a system, more sophisticated, *active* control mechanisms must be provided. These take the form of so-called feedback loops, which are of equal importance in electrical engineering and in the physiology of all living beings, including unicellular organisms. Fig. 14 shows

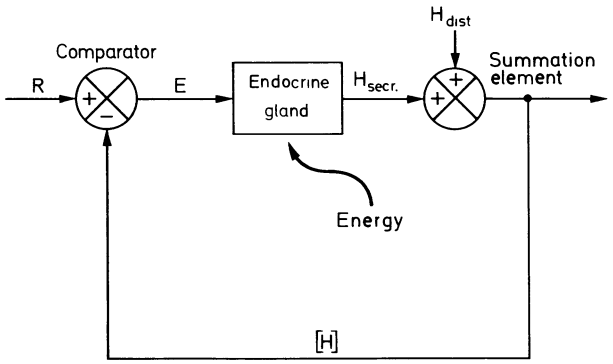


Fig. 14. Hypothetical endocrine system with negative feedback. The arrows indicate the direction of the signal flow. The symbols are explained in the text

a greatly simplified endocrine system with feedback control. Like all subsequent examples of physiological control systems, it is purely hypothetical and represents a simplification of the true conditions. This will apply in particular to the transfer functions that we shall assume to be linear, which is, however, only exceptionally true of physiological systems. The following symbols apply to Fig. 14:

H_{secr} = hormone secretion rate;
H_{dist} = external disturbance of system e.g. injection of exogenous hormone;

* I am grateful to Dr. ROLF ARNDT, Zurich, for his help in preparing this section.

$[H]$ = feedback signal, in this case hormone concentration which is a simple function of $H_{secr} + H_{dist}$;
R = reference input;
E = error signal.

We now apply the transfer functions of Fig. 15 to the control system of Fig. 14. The transfer function on the left indicates that simple subtraction of the feedback signal $[H]$ from the reference value R occurs at the comparator. The resulting signal E is converted into hormone secretion in the endocrine gland according to the transfer function on the right. Thus we have built a simple system with negative feedback and the hormone level in the blood will become stabilized at the reference value. Most endocrine systems are of course more complicated. In the control of the thyroid gland the error signal E acts on pituitary thyrotropin release. The latter process, however, is also under hypothalamic control by thyrotropin releasing hormone. We therefore have an additional transfer function in this system. Such complex systems retain negative feedback characteristics, provided the error signal abolishes or diminishes the effect of an external disturbance. Physiological feedback systems often function without apparent comparator elements and without reference values. We can cite the control of blood sugar by insulin as an example symbolized by the simple feedback loop of Fig. 16a. Despite the fact that comparator and reference values appear to be absent, the system possesses a functioning negative feedback. The desired blood levels of sugar and insulin are not determined by a reference input fed in from an external source, but derived from the equilibrium point of the entire system. This point corresponds to the point of intersection of the transfer function curves presented in a single diagram (Fig. 16b). In this example, the reference input is implicitly contained in the transfer functions.

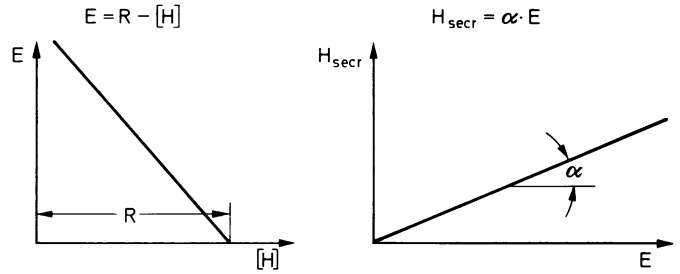


Fig. 15. Transfer functions of the system in Fig. 14. Left, transfer function of the comparator, and right, transfer function of the endocrine gland. α = constant. Meaning of remaining symbols as in Fig. 14

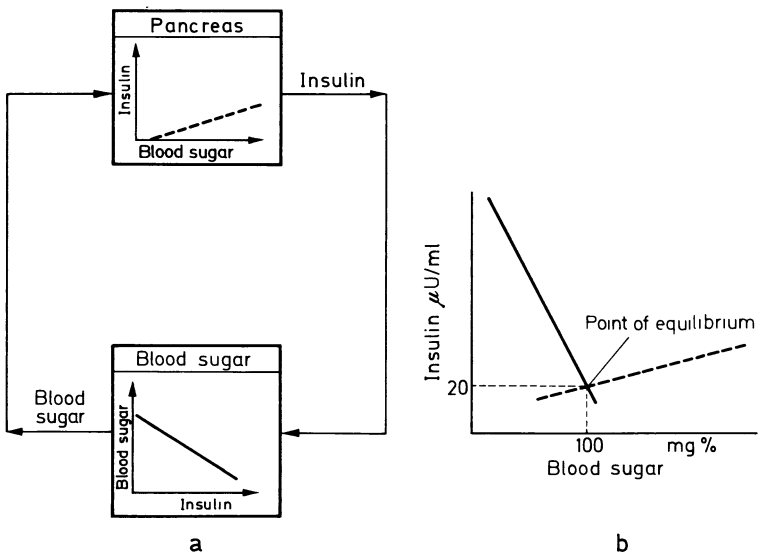


Fig. 16a and b. Control of blood-sugar level by insulin. a Diagram of a simple system with negative feedback. The transfer functions are indicated in the elements of the system. b Transfer functions of Fig. 16a drawn into a single system of coordinates. The point of intersection of the transfer functions gives the point of equilibrium of the control system. It corresponds to and replaces the reference value

Displacement of one transfer function also results in a change of the set-point of the system.

External energy must be supplied to any control system at least at one point of the feedback loop (see Fig. 14). This energy is required to amplify signals passing through the loop at one or several sites. Without this amplification of signals the feedback would be inoperative and the system would react to a disturbance like a system with passive control. To illustrate this statement we shall return to the example of blood-sugar regulation by insulin (Fig. 16), and disturb the system by infusing glucose. The blood-sugar level will change as shown in Fig. 17, where the following symbols are used:

ΔBS = theoretic elevation of blood sugar in the absence of feedback, e.g. following interruption of the feedback loop.

Δbs = actual elevation of blood sugar in the presence of functioning feedback, so-called residual disturbance.

The ratio $\frac{\Delta bs}{BS}$ is called minification. It is a good measure of the efficiency of a control system, since it indicates to what degree an external disturbance is compensated for by the feedback. The following relation is then valid:

$$\text{Minification} = \frac{\Delta bs}{BS} = \frac{1}{1 + A}\,*,$$

where A = signal amplification during passage through the whole loop;
= homeostatic index.

If $A = 0$, then the minification is 1. Any disturbance is therefore able to exert its full effect and the system behaves like a passive system without feedback.

If $A = \infty$, the minification is 0, and a disturbance is completely compensated for in the control system.

All the feedback systems mentioned so far have a *negative* feedback, i.e. the error signal works the opposite way to an external disturbance and diminishes its effects. For the sake of completeness, it must be mentioned here that systems with *positive* feedback are known to occur in electrical engineering as well as in physiology. They can be defined as unstable systems, since an external disturbance is amplified by the feedback and in theory grows infinitely. Infinite amplification of the disturbance is of course limited in fact by the amount of energy supplied externally and by other physiological factors of the system.

Systems with negative feedback can also become unstable when they begin to oscillate under certain conditions. Usually such oscillations are quickly attenuated, as is indicated for the blood-sugar curve shown in Fig. 17. Such

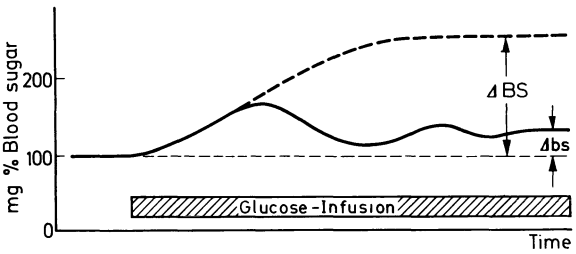


Fig. 17. Behavior of the blood-sugar level during an infusion of glucose, in the system shown in Fig. 16. The full line shows the behavior with functioning feedback, the dashed line shows the behavior with interrupted feedback, e.g. due to pancreatectomy. In the latter case the system reacts like one with passive control. See text for meaning of symbols

oscillations are due mainly to the *inertia* or time delay of a system. This delay is defined as the time needed for a signal to pass along the whole of the feedback loop. Returning to Fig. 14, we now postulate that in the endocrine gland the error signal E is converted into hormone release only after a delay of t seconds, because hormone biosynthesis requires a certain finite time. Hormone concentration $[H]$ will now always lag behind the error signal E by t seconds, as can be seen in Fig. 18 (two lower curves). Since the comparator element still executes the operation $E = R - (H)$ [or $R = E + (H)$], the system can start oscillating as shown in Fig. 18, where E and $[H]$ show a phase difference of 180°. The mathematical operation $E + [H]$ performed by the comparator always gives the required reference value R at any given time. In spite of the constant reference input our endocrine system now oscillates due to the postulated inertia of the endocrine cell*. Such oscillations arise in various physiological systems and even in unicellular organisms and in cell-free extracts. The phenomenon may be of importance in endocrinology since it may explain the female menstrual cycle. Although it is generally assumed that the periodicity of menstruation is controlled through a "clock" mechanism of the central nervous system, MILHORN (1966) put forward the idea that the menstrual cycle is the result of slow oscillations of a feedback system. ODELL's (1968)

* Refer to textbooks on control theory for the derivation of this formula.

* A more detailed analysis of conditions leading to oscillations of a system can be found in J. H. MILSUM (1966).

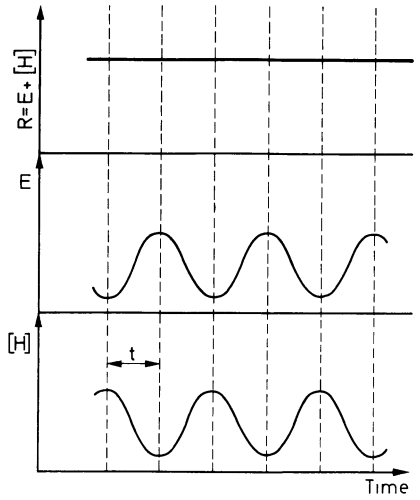


Fig. 18. Oscillations in the control system shown in Fig. 14. It has been postulated that the error signal E is converted into hormone secretion in the endocrine gland only after a latent period of t seconds. Thus, hormone release always lags behind the error signal E by t seconds, as can be seen in the two lower curves. The comparator executes the operation $E = R - [H]$ (or $R = E + [H]$), which gives the required constant reference input for any chosen time (upper curve). Meaning of symbols as in Fig. 14

experiments provide a good argument for this. He demonstrated that the periodicity of gonadotropin secretion disappears completely in castrated women. This may be taken to indicate that there is no central "clock" mechanism. Loss of the ovaries corresponds to interruption of the feedback loop. If the loop was artificially closed by administration of estradiol and progesterone in ODELL's experiments periodic release of gonadotropins recurred, indicating that the system had begun to oscillate again.

A number of endocrine control systems have now been mathematically analyzed and the corresponding model systems have been designed. The model of YATES (1968) for ACTH and corticosteroid secretion, which contains several feedback loops, has been tested in an analog computer and its performance so far has shown very good agreement to the physiologic system. Models of the control of ovulation in women (N. B. SCHWARTZ, 1969) and in rats (VAN DE WIELE, 1970) have also been published. It may be argued that these models are of no great practical value except for the amusement of the investigator. However N. B. SCHWARTZ (1969) has correctly pointed out that the mathematical formulation of the processes of endocrine feedback control has forced experimental endocrinologists to treat their data on a more exact and rational basis, instead of adopting the old semiquantitative and intuitive approach. The models have also helped in the planning of specific experiments to test assumptions made in the course of the model building. Although endocrine feedback model systems have so far had few consequences for the field of clinical endocrinology, any clinician looking at the models mentioned above will concede that endocrine feedback control is vastly more complicated than is usually thought. There is no doubt that the knowledge gained by these models will one day influence clinical endocrinology.

References

ARIENS, E. J.: Receptor theory and structure-action relationships. Advanc. Drug Res. **3**, 235 (1966).

— ROSSUM, J. M. VAN, SIMONIS, A. M.: A theoretical basis of molecular pharmacology. Arzneimittel-Forsch. **6**, 282 (1956).

BALLY, P. R.: Aspects of glucagon-induced lipolysis in adipose tissue. In: Pharmacology of hormonal polypeptides and proteins, ed. BACK, MARTINI and PAOLETTI, p. 416. New York: Plenum Press 1968.

BARNETT, C. A., WICKS, W. D.: Regulation of phosphoenolpyruvate carboxykinase and tyrosine transaminase in hepatoma cell cultures. J. biol. Chem. **246**, 7201 (1971).

BAULIEU, E.-E., WIRA, C. R., MILGROM, E., RAYNAUD-JAMMET, C.: Ribonucleic acid synthesis and oestradiol action in the uterus. Acta endocr. (Kbh.) **71**, Suppl. 168, p. 396 (1972).

BAXTER, J. D., ROUSSEAU, G. G., BENSON, M. C., GARCEA, R. L., ITO, J., TOMKINS, G. M.: Role of specific cytoplasmic receptors in glucocorticoid action. Proc. nat. Acad. Sci. (Wash.) **69**, 1892 (1972).

BEGG, D. J., MUNRO, H. N.: Action of thyroid-stimulating hormone on ribonucleic acid synthesis in thyroid slices and in isolated thyroid nuclei. Nature (Lond.) **207**, 483 (1965).

BIRNBAUMER, L., RODBELL, M.: Adenyl cyclase in fat cells. III. Hormone receptors. J. biol. Chem. **244**, 3477 (1969).

BOWNESS, J. M.: Epinephrine: cascade reactions and glycogenolytic effect. Science **152**, 1370 (1966).

BRETSCHER, M. S.: How repressor molecules function. Nature (Lond.) **217**, 509 (1968).

BUSH, I. E.: Chemical and biological factors in the activity of adrenocortical steroids. Pharmacol. Rev. **14**, 317 (1962).

BUTCHER, R. W., HO, R. J., MENG, H. C., SUTHERLAND, E. W.: Adenosine 3',5'-monophosphate in biological materials. II. The measurement of adenosine 3',5'-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. J. biol. Chem. **240**, 4515 (1965).

CHANCE, R. E., ELLIS, R. M., BROMER, W. W.: Porcine proinsulin: characterization and amino acid sequence. Science **161**, 165 (1968).

CLEVER, U., KARLSON, P.: Induktion von Puffveränderungen in den Speicheldrüsenchromosomen von Chironomus tentans durch Ecdyson. Exp. Cell Res. **20**, 623 (1960).

CLYNES, M.: Unidirectional rate sensitivity: a biocybernetic law of reflex and humoral systems as physiologic channels of control and communication. Ann. N.Y. Acad. Sci. **92**, 946 (1961).

COHEN, P. P.: Biochemical differentiation during amphibian metamorphosis. Science **168**, 533 (1970).

CROFFORD, O. B.: The uptake and inactivation of native insulin by isolated fat cells. J. biol. Chem. **243**, 362 (1968).

— RENOLD, A. E.: Glucose uptake by incubated rat epididymal adipose tissue. Characteristics of the glucose transport system and action of insulin. J. biol. Chem. **240**, 3237 (1965).

CUATRECASAS, P.: Interaction of insulin with the cell membrane: The primary action of insulin. Proc. nat. Acad. Sci. (Wash.) **63**, 450 (1969).
— Unmasking of insulin receptors in fat cells and fat cell membranes. Perturbation of membrane lipids. J. biol. Chem. **246**, 6532 (1971a).
— Properties of the insulin receptor of isolated fat cell membranes. J. biol. Chem. **246**, 7265 (1971b).
— Insulin-receptor interactions in adipose tissue cells: Direct measurement and properties. Proc. nat. Acad. Sci. (Wash.) **68**, 1264 (1971c).
— Properties of the insulin receptor isolated from liver and fat cell membranes. J. biol. Chem. **247**, 1980 (1972).
DALLMAN, M. F., YATES, F. E.: Dynamic asymmetries in the cortico-steroid feedback path and distribution-binding-metabolism elements of the adrenocortical system. Ann. N.Y. Acad. Sci. **156**, 696 (1969).
DE MOOR, P., STEENO, O., HEYNS, W.: Possible role or significance of protein-steroid binding in plasma. Ann. Endocr. (Paris) **29**, 119 (1968), Suppl.
EDELMANN, I. S., BOGOROCH, R., PORTER, G. A.: On the mechanism of action of aldosterone on sodium transport: the role of protein synthesis. Proc. nat. Acad. Sci. (Wash.) **50**, 1169 (1963).
FREYCHET, P., KAHN, R., ROTH, J., NEVILLE, D. M.: Insulin interactions with liver plasma membranes. Independence of binding of the hormone and its degradation. J. biol. Chem. **247**, 3953 (1972).
GELEHRTER, T. D., TOMKINS, G. M.: Posttranscriptional control of tyrosine aminotransferase synthesis by insulin. Proc. nat. Acad. Sci. (Wash.) **66**, 390 (1970).
GILL, G. N., GARREN, L. D.: Role of the receptor in the mechanism of action of adenosine 3',5'-cyclic monophosphate. Proc. nat. Acad. Sci. (Wash.) **68**, 786 (1971).
GLIEMANN, J.: Insulin-like activity of dilute human serum assayed by an isolated adipose cell method. Diabetes **14**, 643 (1965).
GOLDBERG, N. D., VILLAR-PALASI, C., SASKO, H., LARNER, J.: Effects of insulin treatment on muscle 3',5'-cyclic adenylate levels in vivo and in vitro. Biochem. biophys. Acta (Amst.) **148**, 665 (1967).
GRAHAME-SMITH, D. G., BUTCHER, R. W., NEY, R. L., SUTHERLAND, E. W.: Adenosine 3',5'-monophosphate as the intracellular mediator of the action of adrenocorticotropic hormone on the adrenal cortex. J. biol. Chem. **242**, 5535 (1967).
GREENMAN, D. L., WICKS, W. D., KENNEY, F. T.: Stimulation of ribonucleic acid synthesis by steroid hormones. II. High molecular weight components. J. biol. Chem. **240**, 4420 (1965).
GRISWOLD, M. D., COHEN, P. P.: Alteration of deoxyribonucleic acid-dependent ribonucleic acid polymerase activities in amphibian liver nuclei during thyroxine-induced metamorphosis. J. biol. Chem. **247**, 353 (1972).
HALL, R., TUBMEN, J.: Effect of thyroid-stimulating hormone on ribonucleic acid synthesis in the calf thyroid in vitro in the presence of Puromycin. J. biol. Chem. **243**, 1598 (1968).
HAMILTON, T. H., WIDNELL, C. C., TATA, J. R.: Synthesis of ribonucleic acid during early estrogen action. J. biol. Chem. **243**, 408 (1968).
HOCH, F. L.: Biochemical actions of thyroid hormones. Physiol. Rev. **42**, 605 (1962).
— Early action of injected l-Thyroxine on mitochondrial oxidative phosphorylation. Proc. nat. Acad. Sci. (Wash.) **58**, 506 (1967).
ILLIANO, G., CUATRECASAS, P.: Modulation of adenylate cyclase activity in liver and fat cell membranes by insulin. Science **175**, 906 (1972).
INGBAR, S. H.: The interaction of the thyroid hormones with proteins of human plasma. Ann. N.Y. Acad. Sci. **86**, 440 (1960).
JENSEN, E. V., JACOBSON, H. I.: Basic guides to the mechanism of estrogen action. Recent Progr. Hormone Res. **18**, 387 (1962).
— DE SOMBRE, E. R.: Mechanism of action of the female sex hormones. Ann. Rev. Biochem. **41**, 203 (1972).
— SUZUKI, T., KAWASHIMA, T., STUMPF, W. E., JUNGBLUT, P. W., DE SOMBRE, E. R.: A two step mechanism for the interaction of estradiol with rat uterus. Proc. nat. Acad. Sci. (Wash.) **59**, 632 (1968).
KARLSON, P.: Biochemische Wirkungsweise der Hormone. Dtsch. med. Wschr. **86**, 668 (1961).
KATZENELLENBOGEN, B. S., GORSKI, J.: Estrogen action in vitro. Induction of the synthesis of a specific uterine protein. J. biol. Chem. **247**, 1299 (1972).
KORNER, A.: Growth hormone regulation of biosynthesis of protein and ribonucleic acid. Recent Progr. Hormone Res. **21**, 205 (1965).
— Hormonal control of protein synthesis. Proc. roy. Soc. B **176**, 287 (1970).
KRISHNA, G., HYNIE, S., BRODIE, B. B.: Effects of thyroid hormones on adenyl cyclase in adipose tissue and on free fatty acid mobilization. Proc. nat. Acad. Sci. (Wash.) **59**, 884 (1968).
LABRIE, F., LEMAIRE, S., POIRIER, G., PELLETIER, G., BOUCHER, R.: Adenohypophyseal secretory granules. I. Their phosphorylation and association with protein kinase. J. biol. Chem. **246**, 7311 (1971).
LACY, P. E.: The pancreatic beta cell. Structure and function. New Engl. J. Med. **276**, 187 (1967).
— HOWELL, S. L., YOUNG, D. A., FINK, C. J.: Events in the secretion of Insulin. Nature (Lond.) **219**, 1177 (1968).
LAMBERT, A. E., JEANRENAUD, B., RENOLD, A. E.: Enhancement by caffeine of glucagon-induced and tolbutamide-induced insulin release from isolated foetal pancreatic tissue. Lancet **1967**[1], 819 (1967).
LEFKOWITZ, R. J., ROTH, J., PRICER, W., PASTAN, I.: ACTH-receptors in the adrenal: Specific binding of ACTH-^{125}I and its relation to adenyl cyclase. Proc. nat. Acad. Sci. (Wash.) **65**, 745 (1970).
LERNER, L. J.: Hormone antagonists: Inhibition of specific activities of estrogen and androgen. Recent Progr. Hormone Res. **20**, 435 (1964).
LEVEY, G. S., EPSTEIN, S. E.: Myocardial adenyl cyclase activation by thyroid hormones and evidence for two adenyl cyclase systems. J. clin. Invest. **48**, 1663 (1969).
LEVINE, R., GOLDSTEIN, M. S.: On the mechanism of action of insulin. Recent Progr. Hormone Res. **11**, 343 (1955).
LIAO, S.: Influence of testosterone on template activity of prostatic ribonucleic acids. J. biol. Chem. **240**, 1236 (1965).
MACH, B., REICH, E., TATUM, E. L.: Separation of the biosynthesis of the antibiotic polypeptide Tyrocidine from protein biosynthesis. Proc. nat. Acad. Sci. (Wash.) **50**, 175 (1963).
MAKMAN, M. H., DVORKIN, B., WHITE, A.: Influence of cortisol on the utilization of precursors of nucleic acids and protein by lymphoid cells in vitro. J. biol. Chem. **243**, 1485 (1968).
— NAKAGAWA, S., WHITE, A.: Studies of the mode of action of adrenal steroids on lymphocytes. Recent Progr. Hormone Res. **23**, 195 (1967).
MALAISSE, W. J., MALAISSE-LAGAE, F., MAYHEW, D.: A possible role for the adenylcyclase system in insulin secretion. J. clin. Invest. **46**, 1724 (1967).
MCCARTHY, K., LOVENBERG, W., SJOERDSMA, A.: The mechanism of inhibition of horse liver alcohol dehydrogenase by thyroxine and related compounds. J. biol. Chem. **243**, 2754 (1968).
MILHORN, H. T.: The application of control theory to physiological systems. Philadelphia: W. B. Saunders 1966.
MILSUM, J. H.: Biological control systems analysis, p. 45. New York: McGraw Hill 1966.

MONOD, J.: The Upjohn Lecture of the Endocrine Society. On the mechanism of molecular interactions in the control of cellular metabolism. Endocrinology **78**, 412 (1966).

MONOD, J. T., WYMAN, T., CHANGEUX, J.: On the nature of allosteric transitions: A plausible model. J. molec. Biol. **12**, 88 (1965).

NEUMANN, F., BERSWORDT-WALLRABE, R. VON, ELGER, W., STEINBECK, H.: Hormonhemmer — Untersuchungen mit Testosteron-Antagonisten. In: Wirkungsmechanismen der Hormone. Bearbeitet von P. KARLSON, p. 218. Berlin-Heidelberg-New York: Springer 1967.

NICOLOFF, J. T., DOWLING, J. TH., PATTON, D. D.: Inheritance of decreased thyroxine-binding by the thyroxine-binding globulin. J. clin. Endocr. **24**, 294 (1964).

ODELL, W. D., SWERDLOFF, R. S.: Progestogen-induced luteinizing and follicle-stimulating hormone surge in postmenopausal women: a simulated ovulatory peak. Proc. nat. Acad. Sci. (Wash.) **61**, 529 (1968).

O'MALLEY, B. W., MCGUIRE, W. L., KOHLER, P. O., KORENMAN, S. G.: Studies on the mechanism of steroid hormone regulation of synthesis of specific proteins. Recent Progr. Hormone Res. **25**, 105 (1969).

— ROSENFELD, G. C., COMSTOCK, J. P., MEANS, A. R.: Induction of specific translatable messenger RNA's by oestrogen and progesterone. Acta endocrinol. **71**, Suppl. 168, p. 381 (1972).

OPPENHEIMER, J. H.: Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. New Engl. J. Med. **278**, 1153 (1968).

– SQUEF, R., SURKS, M. I., HAUER, H.: Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. J. clin. Invest. **42**, 1769 (1963).

OTT, F. T., RECANT, L.: Effects of N-Ethyl Maleimide and insulin upon adipose tissue. Proc. Soc. exp. Biol. (N.Y.) **112**, 880 (1963).

PARK, C. R., REINWEIN, D., HENDERSON, M. J., CADENAS, E., MORGAN, H. E.: The action of insulin on the transport of glucose through the cell membrane. Amer. J. Med. **26**, 674 (1959).

PENA, A., DVORKIN, B., WHITE, A.: Effects of a single injection of cortisol on amino acid-incorporating activities of rat liver and thymic preparations in vitro. J. biol. Chem. **241**, 2144 (1966).

REICHLIN, S., UTIGER, R. D.: Regulation of pituitary-thyroid axis in man: Relationship to concentration of free and total thyroxine in plasma. J. clin. Endocrin. **27**, 251 (1967).

ROBBINS, J., RALL, J. E.: Proteins associated with the thyroid hormones. Physiol. Rev. **40**, 415 (1960).

ROBISON, G. A., BUTCHER, R. W., SUTHERLAND, E. W.: Adenylcyclase as an adrenergic receptor. Ann. N.Y. Acad. Sci. **139**, 703 (1967).

— — — Cyclic AMP. Ann. Rev. Biochem. **37**, 149 (1968).

RODBELL, M., BIRNBAUMER, L., POHL, S. L., KRANS, H. M. J.: Glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanyl nucleotides in glucagon action. J. biol. Chem. **246**, 1877 (1971c).

— — — SUNDBY, F.: The reaction of glucagon with its receptor: Evidence for discrete regions of activity and binding in the glucagon molecule. Proc. nat. Acad. Sci. (Wash.) **68**, 909 (1971d).

— KRANS, H. M. J., POHL, S. L., BIRNBAUMER, L.: Glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. III. Binding of glucagon: Method of assay and specificity. J. biol. Chem. **246**, 1861 (1971a).

— — — — Glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. IV. Effects of guanyl nucleotides on binding of ^{125}I-glucagon. J. biol. Chem. **246**, 1872 (1971b).

SAHIB, M. K., JOST, Y.-C., JOST, J.-P.: Role of cyclic adenosine 3',5'-monophosphate in the induction of hepatic enzymes. J. biol. Chem. **246**, 4539 (1971).

SANDBERG, A. A., ROSENTHAL, H., SCHNEIDER, S. L., SLAUNWHITE, W. R., JR.: Protein-steroid interactions and their role in the transport and metabolism of steroids. In: Steroid dynamics, ed. G. PINCUS, p. 1. New York: Academic Press 1966.

SCATCHARD, G.: The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. **51**, 660 (1949).

SCHWARTZ, A. G., AMOS, H.: Insulin dependence of cells in primary culture: Influence on ribosome integrity. Nature (Lond.) **219**, 1366 (1968).

SCHWARTZ, I. L., HECHTER, O.: Insulin structure and function. Reflections on the present state of the problem. Amer. J. Med. **40**, 765 (1966).

SCHWARTZ, N. B.: A model for the regulation of ovulation in the rat. Recent Progr. Hormone Res. **25**, 1 (1969).

SEELIG, S., SAYERS, G., SCHWYZER, R., SCHILLER, P.: Isolated adrenal cells: $ACTH_{11-24}$, a competitive antagonist of $ACTH_{1-39}$ and $ACTH_{1-10}$. FEBS Letters **19**, 239 (1971).

SEKERIS, C. E.: Wirkung der Hormone auf den Zellkern. In: Wirkungsmechanismen der Hormone. Bearbeitet von P. KARLSON, p. 126. Berlin-Heidelberg-New York: Springer 1967.

SHARP, G. W. G., LEAF, A.: Mechanism of action of Aldosterone. Physiol. Rev. **46**, 593 (1966).

SOKOLOFF, L., ROBERTS, P. A., JANUSKA, M. M., KLINE, J. E.: Mechanism of stimulation of protein synthesis by thyroid hormones in vivo. Proc. nat. Acad. Sci. (Wash.) **60**, 652 (1968).

SOSKIN, S., LEVINE, R.: A relationship between the blood sugar level and the rate of sugar utilization, affecting the theories of diabetes. Amer. J. Physiol. **120**, 761 (1937).

STEINER, D. F., CUNNINGHAM, D., SPIGELMAN, L., ATEN, B.: Insulin biosynthesis: evidence for a precursor. Science **157**, 697 (1967).

STUMPF, W. H.: Subcellular distribution of ^{3}H-estradiol in rat uterus by quantitative antoradiography — A comparison between ^{3}H-estradiol and ^{3}H-norethynodrel. Endocrinology **83**, 777 (1968).

SUTHERLAND, E. W., RALL, T. W.: Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J. biol. Chem. **232**, 1077 (1958).

— ROBISON, G. A.: Metabolic effects of catecholamines. Pharmacol. Rev. **18**, 145 (1966).

TAPLEY, D. F., KIMBERG, D. V., BUCHANAN, J. L.: The mitochondrion. New Engl. J. Med. **276**, 1182 (1967).

TATA, J. R.: Hormonal regulation of growth and protein synthesis. Nature (Lond.) **219**, 331 (1968).

THOMPSON, E. B., TOMKINS, G. M., CURRAN, J. F.: Induction of Tyrosine alpha-ketoglutarate transaminase by steroid hormones in a newly established tissue culture cell line. Proc. nat. Acad. Sci. (Wash.) **56**, 296 (1966).

TOFT, D., GORSKI, J.: A receptor molecule for estrogens: Isolation from the rat uterus and preliminary characterization. Proc. nat. Acad. Sci. (Wash.) **55**, 1574 (1966).

TOMKINS, G. M., MAXWELL, E. S.: Some aspects of steroid hormone action. Ann. Rev. Biochem. **32**, 677 (1963).

TURKINGTON, R. W.: Induction of milk protein synthesis by placental lactogen and prolactin in vitro. Endocrinology **82**, 575 (1968).

— BREW, K., VANAMAN, TH. C., HILL, R. L.: The hormonal control of lactose synthetase in the developing mouse mammary gland. J. biol. Chem. **243**, 3382 (1968).

VALLOTTON, M. B.: Operational definition of the hormone. Helv. med. Acta **35**, 459 (1969/70).

VANDE WIELE, R. L., BOGUMIL, J., DYRENFURTH, I., FERIN, M., JEWELEWICZ, R., WARREN, M., RIZKALLAH, T., MIKHAIL, G.: Mechanisms regulating the menstrual cycle in women. Recent Progr. Hormone Res. **26**, 63 (1970).

WALSH, D. A., PERKINS, J. P., BROSTROM, C. O., HO, E. S., KREBS, E. G.: Catalysis of the phosphorylase kinase activation reaction. J. biol. Chem. **246**, 1968 (1971).

WALTER, R., RUDINGER, J., SCHWARTZ, I. L.: Chemistry and structure-activity relations of the antidiuretic hormones. Amer. J. Med. **42**, 653 (1967).

WICKS, W. D., GREENMAN, D. L., KENNEY, F. T.: Stimulation of ribonucleic acid synthesis by steroid hormones. I. Transfer RNA. J. biol. Chem. **240**, 4414 (1965).

WILBRANDT, W., ROSENBERG, TH.: The concept of carrier transport and its corollaries in pharmacology. Pharmacol. Rev. **13**, 109 (1961).

WOLFF, J.: The effect of thyroxine on isolated dehydrogenases. II. Sedimentation changes in glutamic dehydrogenase. J. biol. Chem. **237**, 230 (1962).

WOOL, I. G., CAVICCHI, PH.: Insulin regulation of protein synthesis by muscle ribosomes: effect of the hormone on translation of messenger RNA for a regulatory protein. Proc. nat. Acad. Sci. (Wash.) **56**, 991 (1966).

— MUNRO, A. J.: An influence of insulin on the synthesis of a rapidly labeled RNA by isolated rat diaphragm. Proc. nat. Acad. Sci. (Wash.) **50**, 918 (1963).

WURTMAN, R. J.: Catecholamines. New Engl. J. Med. **273**, 637 (1965).

YATES, F. E., BRENNAN, R. D., URQUHART, J., DALLMAN, M. F., LI, C. C., HALPERN, W.: A continuous system model of adrenocortical function. In: Systems theory and biology, ed. M. D. MESAROVIC, p. 141. Berlin-Heidelberg-New York: Springer 1968.

II. The Hypothalamus

A. LABHART

With Contributions by

CHR. HEDINGER, G. TÖNDURY, and G. KISTLER

A. Definition

The hypothalamus has a diameter of only 2.5 cm and makes up 1/300th of the weight of the brain. Nevertheless, this tiny organ coordinates and regulates most of the vital processes of life. Modulations are effected through neural and neuro-endocrine routes. The schema of HARRIS (Fig. 1) shows how the humoral substances secreted by neurons can affect the endorgans, with or without intervention of the endocrine system. The physiologic and clinical aspects of the hypothalamus will only be discussed in relation to their influence on the endocrine system, as befits a textbook of endocrinology.

The hypothalamus controls the endocrine system primarily through neurosecretions acting on the anterior pituitary. These are usually of a stimulatory nature (releasing factors), but may occasionally be of an inhibitory nature (inhibitory factors). However, in contrast to the neurohypophysis, there is only a conditioned dependence between the anterior pituitary and the hypothalamus, since the anterior pituitary can also produce hormones independently of the hypothalamus. It is probable that the anterior pituitary is also capable of certain autonomic control. On the other hand, the hypothalamo-neurohypophyseal system forms a uniform endocrine organ which is concerned with the regulation of the osmosis and volume of the body fluids. This system is discussed in a separate chapter.

B. Embryology and Gross Anatomy

G. TÖNDURY and G. KISTLER

In the human embryo, the anlagen of the forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon) become distinguishable even before closure of the rostral end of the neural groove. When the brain becomes a closed tube, these three subdivisions develop into the primary brain vesicles. Early in the fourth week (when the length of the embryo is approximately 3 mm) a dorsal groove forms in the prosencephalic vesicle subdividing it into the telencephalon, with the primitive cerebral hemispheres, and the diencephalon, with the anlage of the eyes, i.e., the optic vesicles. In embryos 7–8 mm in length, the anlagen of the pineal gland (epiphysis) and the neurohypophysis appear as small outgrowths of the roof and the floor of the diencephalon, respectively.

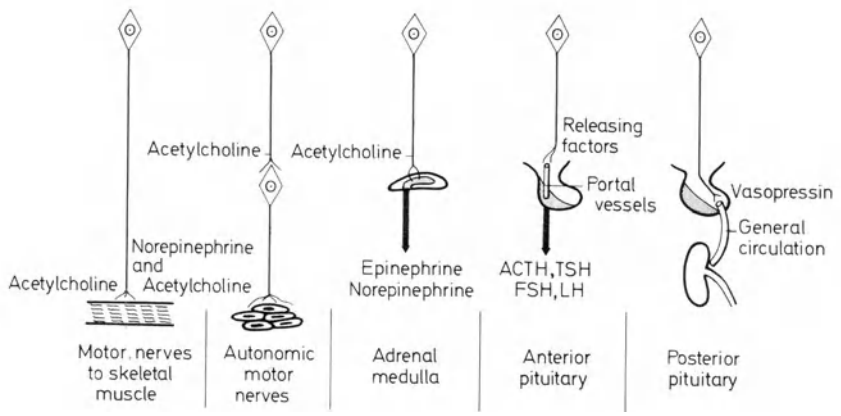


Fig. 1. Five situations in which humoral substances are secreted by neurons. The substances liberated in each case are indicated beside the nerve endings. (After HARRIS, from GANONG, in MARTINI and GANONG, 1966)

The Diencephalon can be divided into four more or less distinct major parts. The dorsally situated *epithalamus* (1) comprises the epithelial roof of the third ventricle, the striae medullares thalami, the habenular trigones and the pineal gland. The *thalamus* (2) extends basally to the sulcus hypothalamicus Monroi (sulcus diencephalicus ventralis). Its medial and lateral portions are separated from each other by the internal medullary lamina. Both contain a number of morphologically distinct nuclear masses. The *subthalamus* (3) or ventral thalamus is bounded dorsally by the thalamus, ventrally and laterally by the pars peduncularis of the internal capsule, and medially and rostrally by the hypothalamus. It includes the subthalamic nucleus, the zona incerta, the nucleus of the tegmental field of Forel, and, caudally, the rostral parts of both the substantia nigra and the red nucleus. The subthalamus is continuous with the tegmentum and the basilar portions of the mesencephalon and must therefore be considered as part of the extrapyramidal motor system. Because of its abundance of myelinated nerve fibers, the subthalamus can easily be distinguished from the *hypothalamus* (4) which contains mainly nonmyelinated nerve fibers and a much richer vascular network. The mamillary bodies, although usually considered as part of the hypothalamus, belong to the subthalamus. They receive myelinated fibers from the fornix.

The Hypothalamus comprises the lower parts of the ventral walls of the third ventricle as well as the structures of the ventricular floor (chiasma opticum, tuber cinereum and infundibulum). It is separated from the pars telencephalica of the third ventricle by a line connecting the optic chiasm with the posterior edge of the anterior commissure. Caudal and lateral to the hypothalamus is the subthalamus. An inferior view of the brain shows that the hypothalamus is bounded anteriorly by the optic chiasma, laterally by the optic tracts and posteriorly by the mamillary bodies.

The hypothalamus consists of a diffuse matrix of rather small cells which form the central gray substance. Within this matrix, a number of more or less well distinguishable nuclear masses (= nuclei) permit the hypothalamus to be divided morphologically into three regions: The *supraoptic region* (1) lies directly above the optic chiasma. It contains the supraoptic and paraventricular nuclei which are bridged by small groups of cells. Both these nuclei consist of large, often bipolar and dark-staining neurons which show cytoplasmic inclusions of colloidal material and peripherally distributed Nissl substance. The majority of their efferent nerve fibers make up the *supraoptico-hypophyseal tract*, which extends to the posterior lobe of the hypophysis. A minority of the axons, however, terminate within the nuclei themselves or within the infundibulum.

The central gray substance of the *tuberal region* (2) contains the nucleus tuberis infundibularis, the nucleus hypothalamicus ventromedialis and the so-called periventricular area. These nuclear masses consist of small, ovoid cells intermingled with some larger elements. They are the site of origin of the *tubero-hypophyseal tract*, most of whose nerve fibers terminate in close proximity to the specialized blood vessels of the hypophyseal portal system. This in turn extends to the sinusoidal capillary network of the anterior lobe of the hypophysis (see p. 81). Finally, a *lateral area* (3) of the tuber cinereum contains the tubero-mamillary nucleus and the lateral tuberal nuclei. Little is known about their nerve fiber connections.

The Blood Supply of the hypothalamus is ensured mainly by the anterior cerebral artery and the posterior communicating artery. Small branches extend from these vessels to form an intricate network within the nervous parenchyma. The supraoptic and paraventricular areas are characterized by a particularly dense capillary network which is supplied by branches of the anterior cerebral artery, more specifically the supraoptic-paraventricular arteries. The supraoptic nucleus also receives small branches from the superior hypophyseal artery.

The nuclear masses of the tuber cinereum share the general, less dense vascular network of the hypothalamus which is supplied partly by the anterior cerebral arteries and partly by the posterior communicating arteries. The blood supply of this region is therefore ensured by at least two large pairs of arteries which also supply the mamillary bodies.

The hypophysis itself receives its blood supply from the superior and inferior hypophyseal arteries which are branches of the internal carotid (see also p. 40). There are *no direct* connections between the vascular networks of the various hypothalamic nuclei and the hypophysis. The hypothalamic mechanisms controlling the activity of the hypophyseal anterior lobe are discussed on p. 81.

C. Physiology

Exogenous stimuli mediated by the sense organs, such as light and temperature, and endogenous signals from the autonomic nervous system and the psyche, are all received in the hypo-

thalamus by way of brain formations whose functions have been under investigation for the past few years. Complex connections exist between the hypothalamus and phylogenetically older parts of the brain (pyriform cortex, hippocampus, amygdala) and between the hypothalamus and phylogenetically newer parts of the brain (thalamus, mesencephalon) (NAUTA, 1963; DE GROOT, 1966). The pineal body probably also plays a part in this relationship (see p. 73f.). Intracerebral signals are transmitted neurohumorally via many carrier substances. These substances include acetylcholine (cholinesterase), noradrenaline, dopamine, serotonin, and melatonin, all of which are found in varying concentrations (see SHUTE, 1966; MARTINI, 1970; YATES, 1971). These neurotransmitter substances act on the releasing or inhibiting hormone-producing neurons, the "transducer" neurons, which reside in the "hypophysiotropic" area in the ventral hypothalamus. They extend their axons into the median eminence, where the releasing factors are secreted into the capillaries of the primary portal plexus. The neurosecreting neurotransmitters can be influenced by a variety of pharmacologic agents, such as reserpine, phenothiazines, tricyclics and amphetamines, in a complex manner which has not yet been fully explained, so that the releasing hormones and endocrine functions can be manipulated by these drugs (FROHMAN, 1972).

The blood levels of cortisone, thyroxine, triiodothyronine and the gonadal hormones also act as proprioceptive stimuli. They are perceived by the hypothalamus as a negative "feedback" mechanism, and form a connecting link in the controlling system: hypothalamus-anterior pituitary-peripheral endocrine glands.

The precise site of this registration of blood level in the brain has not yet been localized, although stereotaxic lesions and implantations of hormone crystals have been used in attempts at more exact definition. RUF and STEINER have succeeded in using a microelectrophoretic technique to place minute amounts of hormone in the immediate extracellular surroundings of single neurons in the rat's brain. Action currents were registered by microelectrodes with a diameter of 1–3 μm. Injections in the cortex, hippocampus, and thalamus had no effect. On the other hand, neurons in the upper central gray matter around the 3rd ventricle reacted immediately with cessation of activity. The neurons registering the cortisol concentration either produced CRF or functioned as collators controlling other effector neurons (RUF, 1967) (see Chap. VII, p. 299). The feedback mechanism of the individual peripheral hormones is discussed in the chapters dealing with the endocrine organs (see p. 148, 299, 452). It is possible (MARTINI, 1968), but still not certain (MCCANN, 1969), that the pituitary adenotropic hormones also exert a direct "feedback" effect ("short loop") on the hypothalamus (SZENTOGATHAI, 1961).

The hypothalamus receives the exteroceptive and proprioceptive stimuli, registers metabolic processes (osmolality, glucose concentration, the "feedback" mechanism of hormones), integrates and coordinates these factors, and controls seven vital functions via neural and endocrine routes. The functions are:

1. Energy balance,
2. Fluid balance,
3. Heat regulation,
4. Activity and sleep,
5. Circulation and breathing,
6. Growth and maturation,
7. Reproduction.

On one hand, the hypothalamus regulates homeostasis by means of these functions. On the other hand, simple neuroendocrine reflexes (stress-ACTH, suckling reflex-milk ejection, reflex ovulation in certain mammals and birds), or complex neuroendocrine responses to rhythms of long or short duration (day-night, seasonal) also take place within the hypothalamus (ROTHBALLER, 1966).

W. R. HESS has ascribed individual functions to certain areas in the hypothalamus. Thus, destruction of certain regions can cause loss of individual functions, and correspondingly, although rarely, other functions may be stimulated. However, electric, physical and chemical stimuli induce different functions and seldom inhibit them. Atlases are available giving the localization of such experimentally defined areas.

Energy balance and the nutritional intake are regulated by an appetite and satiation center. It is still not certain if these centers respond directly to the arteriovenous difference in blood sugar, to the concentration of free fatty acids, to the insulin content, or to the content of growth hormone in the blood.

Growth and maturation are closely connected with the regulation of energy exchange. Fluid intake and output are closely related to osmoregulation and volume regulation and are controlled by the thirst and osmoregulation centers (FITZSIMONS, 1972). There are areas which can influence sleep, circulation, breathing and body temperature. Finally, maturation, ovarian and testicular function and lactation. which are functions connected with reproduction, are regulated by different areas depending on the animal species. Experimentally-produced

lesions of certain areas can give rise to characteristic syndromes. A summary of these, taken from AKERT, is given in Table 1.

These experimentally definable areas coincide to some extent with morphologically demonstrable formations, but there is a great degree of overlap. HESS denies the concept of single regulation centers. It is not by chance that heat regulation, thyroid function, onset of puberty and ovulation are all controlled by the same area. Endocrine functions are controlled by zones or functional units in the sense of a syncytium rather than by individual neurons each with certain specific actions.

Table 1. Experimentally produced hypothalamic syndromes. (After AKERT, 1959)

	Syndrome	Histological localization	Nature of manifestations 1 = failure 2 = stimulation
Anterior hypothalamus	1. Diabetes insipidus	Supraoptical and paraventricular nuclei	1
	2. Failure of temperature regulation, hyperthermia	Region of the anterior hypothalamic nucleus	1 and 2
	3. Hemorrhagic pulmonary edema	Preoptical medio-ventral area	2
	4. Insomnia, hyperactivity, excitability and manic states	Suprachiasmatic area (?)	2
Tuber cinereum	1. Obesity (hyperphagia)	Region of the ventro-medial hypothalamic nucleus	2
	2. Genital dystrophy	Ventral nucleus of the tuber, infundibular nucleus (?)	1
	3. Pathological inclination to outbursts of temper	Region of the ventro-medial hypothalamic nucleus, and somewhat rostral to this	2
Posterior hypothalamus	1. Hypersomnia, coma	Latero-dorsal zone	1
	2. Poikilothermia	Latero-dorsal zone	1
	3. Anorexia	Lateral hypothalamus adjacent to the cerebral peduncle	1
	4. Pubertas praecox	Infundibular-mammillary region	2 (?)
Ill-defined areas	1. Gastro-intestinal ulcers	Medial basal hypothalamus with extensive longitudinal diffusion	?

The hypothalamus exerts its control on the functions, on the one hand by neurosecretions, and on the other by way of the autonomic nervous system, which also affects the motor nervous system indirectly. Two types of hypothalamic neurosecretion can be differentiated. The paraventricular and supraoptic nuclei produce vasopressin and oxytocin together with a carrier substance, and they are released through the neurohypophysis into the blood of the greater circulation. In addition, polypeptide neurohormones formed or stored in the eminentia mediana reach the blood stream of the pituitary portal system and arrive in the anterior pituitary where they exert a limited local effect (Fig. 2). Fig. 1 (by HARRIS from GANONG, 1969) demonstrates how information from neurons can be transmitted to the endorgans with or without involvement of the endocrine system.

The neurosecretions of the hypothalamo-neurohypophyseal system have been chemically identified and synthesized, and their mode of release is largely known, but we are only beginning to understand the process of hypothalamic regulation of the anterior pituitary by means of neurohormones. It is currently assumed that eight different specifically acting neurohormones are formed within an area of nervous tissue only a few mm in diameter. The existence of other hypothalamic hormones has been suggested but is still not proven. Release of TSH, ACTH, STH, FSH, and LH is stimulated, whereas release of the melanocyte-stimulating hormones (MSH) and prolactin is inhibited. The structures of the thyrotropin-releasing hormone (TRH), luteinizing-hormone releasing hormone (LRH), growth-hormone-releasing hormone (GRH), and melanocyte-stimulating hormone release-inhibiting hormone (MIH) are already known and their synthesis has been accomplished. The existence of a prolactin-inhibiting factor (PIF) is well documented; its nature, however, is not yet known. The existence of GIF (growth-hormone in-

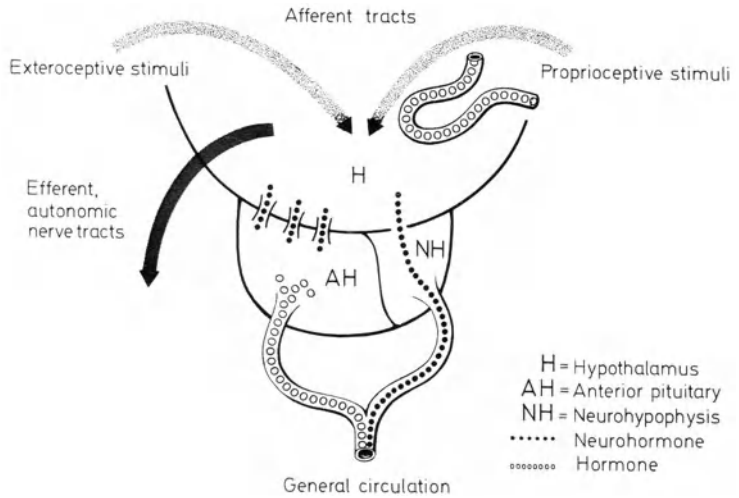


Fig. 2. The hypothalamus as a nervous and neuroendocrine coordination and regulating centre

hibiting factor)*, MRF (melanocyte-stimulating hormone-releasing factor) (McCann, 1969) and PRF (prolactin-releasing factor) has still to be confirmed (Table 2).

Table 2. Neurohormones of the hypothalamus (1973)

Circulating in the anterior pituitary portal system Local actions		Circulating in the greater circulation Distal organic effect
Structure known, compound synthesized		
TRH	Thyrotropin-releasing hormone	
LRH	Luteinizing hormone-releasing hormone	
MIH	Melanocyte-stimulating hormone release inhibiting hormone release-inhibiting hormone	Vasopressin
		Oxytocin
GRH	Growth hormone-releasing hormone	
GIF (GRIH)	Growth hormone release-inhibiting factor	
Existence proven or very probable		
CRF	Corticotropin releasing factor	
FRH	Follicle-stimulating hormone-releasing hormone	
PIF	Prolactin-inhibiting factor	
Existence to be confirmed		
MRF	Melanocyte-stimulating hormone-releasing factor	
PRF	Prolactin-releasing factor	

For more details on the biochemistry, physiology, and pathophysiology of the various hypothalamic releasing hormones see chapters dealing with the different endocrine glands. Whereas the isolation of CRF has not yet been accomplished despite a decade of research (it is still an open question whether the highly purified α_1-CRF, α_2-CRF and β-CRF isolated from neurohypophyses are identical to hypothalamic CRF) (Guillemin, 1964), in 1969 there was a breakthrough in the field of TRH. Out of hundreds of thousands of ovine and porcine hypothalami, it proved possible to extract a few mg of a substance which had a specific TRH-activity *in vivo* in the nanogram range, and *in vitro* in the picogram range. Pyroglu-his-pro-NH_2 (Gillessen, 1970) (Fig. 3) has

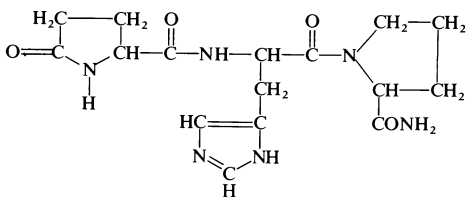


Fig. 3. The thyrotropin releasing hormone

been synthesized; it has practically the same effects and the same physicochemical properties as the purest preparations of TRH from ovine (Burgus, 1970) and porcine (Bowler, 1969) hypothalami, and is apparently identical to TRH. Pyroglutamilhistidylproline-amide is effective when given by the i.v. and i.p. routes in mice and rats, and by the oral and i.v. routes in man, causing a rapid increase in TSH (Hall, 1970). It is inactivated in human plasma at 37° C and is inhibited *in vivo* by triiodothyronine.

Besides nausea, flushing, and a desire to micturate after rapid intravenous injection, no

* In 1973 GIF (GRIH, Somatostatin) has been found to be a decapeptide and has since been synthetized (Schally, 1973; Brazeau, 1973).

serious side effects have been observed. TRH acts specifically on the release of TSH, with the exception of an occasional increase in output of luteinizing hormone and prolactin. TRH allows measurement of the TSH reserve of the pituitary (for different tests and possible therapeutic uses see Chap. VI, p. 242).

LRH is a decapeptide (BABA, 1971) causing a rapid release of LH in animal and man, and to a lesser extent, FSH. Its structure is Pyro–Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2. For tests and therapeutic implications see Chap. IX and X.

GRH has very recently also been shown to be a decapeptide (SCHALLY, 1972) and has already been synthesized (VEBER, 1971). Its diagnostic and therapeutic use has yet to be investigated.

Finally, the structure of MIH has been revealed as tripeptide with the identical amino acids of the C-terminal of oxytocin 1–pro–1–leu–gly–NH_2 (NAIR, 1971).

The neurosecretions controlling the anterior pituitary seem to be formed or accumulated (MARTINI, 1968) in the median eminence, which has led to the suggestion of the term "median eminence-gland" (REICHLIN, 1966).

Receptors regulating the release of pituitary hormones seem to be situated within this small tissue area (MARTINI, 1969). There is evidence that these neurohormones are not only releasing factors, but are also concerned with the formation of hormones in the hypophyseal cells and the growth of these cells. TRH and LRH do not need protein formation for their effect, and it is not blocked by inhibition of RNA or protein synthesis. They act rapidly, mainly by depolarization of the cellular membrane with subsequent Ca^{++} uptake (MCCANN, 1969). There is always a basal secretion of growth hormone, and only its additional release is controlled by the hypothalamus. In contrast, however, the gonadotropic hormones dry up completely in the absence of hypothalamic stimulation. It is assumed that prolactin is regulated through a special mechanism. After section of the hypophyseal stalk, secretion of all the pituitary hormones decreases, with the exception of prolactin which increases and results in galactorrhea. Drugs such as chlorpromazine, which exert an inhibitory effect on the brain stem can also result in the secretion of milk. It is assumed that there is a hypothalamic inhibitory factor "PIF" (prolactin inhibitory factor) (SCHALLY, 1968) whose removal results in a release of prolactin and secretion of milk. It has been stated that there is a prolactin-releasing factor in birds (SCHALLY, 1968). An interesting phylogenetic interpretation is that the hypothalamic releasing factors were originally hormones and regulated the original primitive gut from which the anterior pituitary has developed (HAMMERSCHLAG, 1966).

The hypothalamus forms part of the endocrine system. The anterior pituitary was formerly considered the conductor of the endocrine orchestra but the hypothalamus has now taken over the bâton and the anterior pituitary has been relegated to the position of first violin. The hypothalamus regulates four important endocrine organic systems – the adrenals, the thyroid gland, the gonads, and the growth hormone. This is discussed separately in the appropriate chapters. Only a survey of hypothalamic function within the endocrine system is intended here. The subject of neuroendocrinology is in a state of rapid development and has become an independent specialty beyond the scope of a textbook of endocrinology.

D. Pathology and Clinical Features of Endocrinopathies due to Hypothalamic Involvement

Lesions in the region of the hypothalamus and in neighboring areas can lead to endocrine disorders and syndromes when the hypophysis is not destroyed, as can dysfunction with no morphological substrate.

The diagnosis is difficult and can only seldom be made during life. The lesions may be small and clinically undetectable, or the destruction may be so extensive that neurological symptoms dominate the clinical picture. Furthermore, it is usually only symmetrical lesions that lead to endocrine failure. The early stages of the hypothalamic diseases are better suited to endocrine evaluation, and sometimes only the history indicates that the illness has arisen in the hypothalamus.

The neuroendocrine diseases are most often caused by tumors, such as various types of gliomas, astrocytomas, craniopharyngiomas, ectopic pinealomas, and sometimes suprasellar pituitary adenomas and meningiomas. Inflammatory changes due to basal meningitis and in particular to tuberculosis (COURVOISIER, 1960) and syphilis are now extremely rare. Chronic inflammations, such as granulomas in sarcoidosis, lymphgranulomas, leukemia or thesaurismosis, are also uncommon causes. Damage to the hypothalamus resulting from accidents or vascular aneurysms is also rare.

The symptoms are determined by the localization and the extent of the lesion.

Suprasellar lesions of the infundibulum and the stalk interrupt the hypothalamic control

of the anterior pituitary. See Chap. III, p. 50f. for the disorders of vasopressin and oxytocin secretion. Secretion of the gonadotropins fails completely with the exception of prolactin, which is secreted in increased amounts (failure of PIF, see p. 106). A basal secretion of growth hormone, ACTH, and TSH is retained but is usually uninfluenced by hypothalamic stimuli such as hypo- and hyperglycemia, stress, and variations in the hormonal "feedback" mechanism (inhibition test, methyrapone test).

The clinical picture corresponds to complete or, more frequently, to partial hypopituitarism.

If the lesions are situated high up, as in the case of an ectopic pinealoma, all the axons are interrupted, and their nuclei of origin may be destroyed [*B* in Fig. 4 (ROTHBALLER)]. All the releasing factors are lost. This leads to complete hypogonadism, partial adrenal, thyroid and growth hormone failure, and usually to diabetes insipidus, possibly associated with a simultaneous disturbance of the thirst center.

Deeper-lying lesions such as occur particularly after surgical section of the stalk or with a craniopharyngioma, and occasionally after traumatic lesion of the hypophyseal stalk lead predominantly to destruction of the portal venous system. This usually results in more or less extensive infarction of the anterior pituitary. The releasing factors are still produced, but their relay system is interrupted. The portal system is, however, capable of regeneration providing no foreign bodies are inserted. Neurosecretions continue to be produced. Diabetes insipidus is usually mild, and may be only temporary. The hypopituitarism is of varying severity, usually not very marked, and can regress. The adrenocortical reaction to fever and hypoglycemia can be retained, even when regeneration is hindered by the insertion of metal plates (VAN WYK, 1960).

Partial hypopituitarism, especially secondary hypogonadism, develops in the presence of incomplete lesions [*D* in Fig. 4 (ROTHBALLER)]. The circadian rhythm of the adrenal cortex may be retained or lost. The methyrapone test is sometimes normal and sometimes pathologic.

Galactorrhea, often associated with amenorrhea and uterine atrophy (the Chiari-Frommel syndrome, see p. 592), is characteristic of incomplete infundibular lesions. Failure of the PIF (prolactin inhibiting factor, see p. 106) leads to increased prolactin secretion. A certain basal secretion of growth hormone, ACTH, and gonadotropins must, however, be present for lactation to occur. Galactorrhea typically arises with the introduction of substitution therapy after surgical division of the stalk.

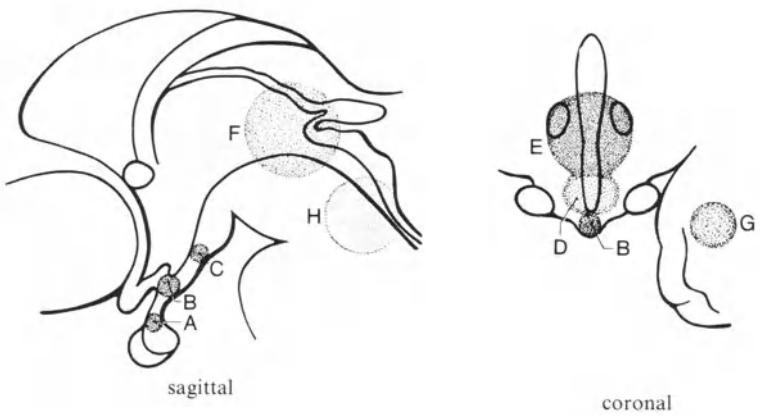


Fig. 4. Lesions of the CNS resulting in endocrinopathies. (After ROTHBALLER, Bull. N. Y. Acad. Med. **42**, 258)

Pressure effects due to the neighboring structures without interruption of the infundibular tracts result in para-infundibular lesions (ROTHBALLER, 1966) [*C* in Fig. 4 (ROTHBALLER)]. This lesion leads to failure of the "feedback" mechanism and loss of homeostasis. Pubertas praecox may then develop (Chap. XIX). These lesions are caused particularly frequently by hamartomas, but any space-occupying processes, including hydrocephalus of the third ventricle, may be causal factors.

The neuroendocrine reflex mechanisms are disturbed in intrahypothalamic lesions. Menstruation becomes disorganized in the presence of ovarian secretion. The gonadotropins are reduced to the greatest extent, TSH to a lesser extent, and ACTH least of all. There is a characteristic combination with disturbances of

emotions, consciousness, energy balance, waking-sleeping rhythm and temperature regulation [*E* in Fig. 4 (ROTHBALLER)]. Lesions outside the hypothalamus, such as lesions of the limbic system or of the brain stem and in the temporal lobes, can also inhibit the circadian rhythm of the adrenocortical secretions. These lesions are often accompanied by epileptic fits. The dexamethasone inhibition test and the methyrapone test are often pathologic. The response of the pituitary-adreno-cortical system to stress is lost in lesions of the afferent nerve tracts or of the stalk, and in particular, in lesions of the reticular formation.

But recently, endocrinopathies with none of the lesions detectable by the morphological methods currently available have become known. Most congenital cases of partial or complete hypopituitarism are probably of hypothalamic origin due to a deficiency of different releasing hormones. Growth-hormone deficiency causing dwarfism (see Chap. V, p. 98) is the most common of this group and ACTH deficiency the rarest. Hypothalamic rather than pituitary origin has to be postulated for many forms of hypgonadotropic hypogonadism (MARTIN, 1972; editorial 1972).

Conversely, as soon as sensitive assays sufficiently sensitive for releasing hormones become available, it will be possible to test the hypothesis that diseases of pituitary hyperfunction, such as certain types of Cushing's syndrome or acromegaly, may result from a primary hypothalamic disturbance.

E. Diagnosis of Hypothalamic Endocrinopathies

Endocrinopathies occurring together with certain neurological manifestations suggest a hypothalamic cause. Homonymous or temporal loss of vision occurs in a third of cases. Atrophy of the optic nerve with central scotomas and damage of the abducens and oculomotor nerves may also occur. Lesions in the anterior hypothalamic region may give rise to the Foster-Kennedy syndrome, characterized by homolateral papillary atrophy, anosmia, and contralateral papilledema. Cranial growth may give rise to impairment of thalamic sensation or even to pyramidal signs. Pleocytosis in the cerebrospinal fluid is suggestive of a pinealoma. Confirmation of the diagnosis is possible by stereotaxic tumor puncture. A series of observations of 100 patients gives an indication of the incidence of neurological disorders of this kind occurring in association with endocrinopathies due to hypothalamic lesions verified at post mortem (BAUER, 1959). Lesions of the hypothalamus are indicated particularly by the following nine symptoms or groups of symptoms: 1. Psychic disorders, especially affective disorders such as acute exogenous reaction type (outbursts of temper, compulsive laughing and crying). 2. Hypersomnia. 3. Obesity due to bulimia. 4. Loss of weight due to lack of appetite. 5. Polydipsia or adipsia. 6. Disorders in temperature regulation (fever or subnormal temperatures). Hypothalamic fever is not accompanied by leukocytosis or malaise, and it responds to sedatives which act on the brain stem. 7. Disorders of the autonomic nervous system (vegetative attacks, autonomic epilepsy). 8. Diabetes insipidus, especially if associated with hypopituitarism. 9. Pubertas praecox (Chap. XIX).

It is, however, often difficult or impossible to differentiate whether such symptoms, in particular hypersomnia, obesity, or loss of weight, are due to organic hypothalamic lesions, or whether changes in character lead to bulimia or anorexia. There are no or only isolated examples of obesity in the human due to hypothalamic lesions which have been verified at post mortem (LIPSETT, 1962).

Various tests can be of use in the differential diagnosis between hypopituitarism, partial hypopituitarism, or hypothalamic endocrinopathies.

The vasopressin test (see p. 388) should theoretically differentiate between failure of the adrenal cortex caused by hypothalamic failure and that caused by pituitary failure until purified CRF preparations are available. If the lesion lies in the hypothalamus, the plasma and urinary levels of corticoids rise under the influence of the vasopressin infusion. This rise does not occur in pituitary insufficiency. The TRH-test and the LRH-test have the same implications.

If the plasma or urinary steroid level is unaltered or not significantly reduced, certain concomitant signs indicate disturbances in the hypothalamic regulation system. These are: a pathological circadian rhythm (KRIEGER, 1966), a pathological dexamethasone inhibition test, and a pathological methyrapone test in the presence of an intact pituitary gland (HOKFELT, 1959; OPPENHEIMER, 1961). Failure of the adrenals to respond to stress, caused for example by a pyrogen injection in the presence of intact pituitary and adrenal glands, and failure of the levels of growth hormone and of the plasma corticoids to rise in response to hypoglycemia (see p. 123), are further indications of a hypothalamic disorder (BETHGE, 1967; LANDON, 1966; GREENWOOD, 1966). Whether the absence of a rise in the aldosterone level in response

to removal of salt is a sign of hypothalamic disorder (HÖKFELT, 1959; KRIEGER, 1966) has yet to be proven. The results of these tests are not only pathological in hypothalamic endocrinopathies, but also in ectopic hormone formation (paraneoplastic endocrine syndrome see Chap. XVI).

Often, however, when the tumors cannot be diagnosed by radiological and isotopic methods, the clinician can only suspect the etiology to be in the hypothalamus. Stereotactic biopsy sometimes provides evidence, otherwise the diagnosis can only be confirmed at operation or from the post-mortem findings. Irradiation and surgery can hardly be considered as therapeutic measures without positive radiological findings. However, where there is endocrine failure such as a partial or complete hypopituitarism due to hypothalamic lesions, substitution therapy must always be instituted.

F. Special Syndromes

1. The Tuberal Form of Dystopia of the Posterior Pituitary Lobe results in the interruption of the hypothalamic neurosecretion into the anterior pituitary, giving rise to hypopituitarism (see p. 104).

2. The Syndrome of Gigantism with acromegalic features occurring during childhood, accompanied by oligophrenia, macrocephaly, and coordination disorders, is assumed to be due to impaired regulation of the secretion of growth hormone resulting from brain injuries sustained at birth (SOTO, 1964). See Chap. XIX for Froehlich's syndrome.

3. The Laurence-Moon-Biedl Syndrome is a degenerative syndrome with inconstant manifestations of endocrine failure, such as diabetes insipidus. Hypogonadism can be of a primary or secondary nature. It is not consistent and is probably due to defects of the central nervous system. The disorder is hereditary, and is probably transmitted through a monohybrid simple recessive gene, though the pathogenesis of the condition is disputed. A reduction in the number of ganglion cells in the hypothalamus was thought to be found in a few of 6 cases confirmed at post mortem.

4. Albright's Syndrome was originally described as polyostotic fibrous dysplasia (Jaffe-Lichtenstein's disease) with pubertas praecox especially in females and patchy skin pigmentation. The bone lesions, often involving facial bones, tend to be unilateral as do the brown, non-elevated skin areas on the same side. Other endocrinopathies have since been observed in this syndrome: goiter and/or hyperthyroidism, acromegaly, Cushing's disease, gynecomastia and acceleration of skeletal growth without overt precocious puberty (HALL, 1972). Premature secretion of FSH and LH has been reported. The euthyroid or toxic goiters show evidence of long-standing hyperplasia without the lymphocytic infiltrations characteristic of Grave's disease but suggestive of abnormal TSH stimulation. True acromegaly has been proven in at least one case, although the facial deformities may mimic the aspect of acromegaly. The observed case of Cushing's disease was of the hypothalamic type. Pituitary histology indicated hyperplasia and no tumor formation. These observations suggest a hypothalamic origin of these endocrinopathies via increased secretion of the corresponding releasing hormones. In the few cases which have been verified at post mortem, the hypothalamus was sometimes found to be intact; however, in one case the mamillary body was smaller than normal, and an additional nucleus was present in the neighboring tissue. Originally the hypothalamic dysfunction was explained by pressure through hyperostoses on the base of the cranium, but although thickening of the cranial base can often been seen radiologically, there are endocrinopathies in cases where the base of the skull is not involved. Thus the endocrine manifestions probably result from a still undetermined hypothalamic disorder.

References

Review Articles

BAJUSZ, E.: Modern trends in neuroendocrinology with special reference to clinical problems. In: BAJUSZ, Clinical neuroendocrinology. Basel-New York: Karger 1967.

— JASSMIN, G.: Major problems in neuroendocrinology. Basel-New York: Karger 1964.

BARGMANN, W., SCHARRER, B. (Editors): Aspects of Neuroendocrinology. Berlin-Heidelberg-New York: Springer 1970.

BAUER, H.G.: Introduction. In: BAJUSZ, Clinical neuroendocrinology. Basel-New York: Karger 1967.

Editorial: Hypothalamic releasing hormones. Brit. med. J. **1972** I, 65.

GANONG, W.F., MARTINI, L.: Frontiers in neuroendocrinology. New York: Oxford University Press 1969.

GUILLEMIN, R.: Hypothalamic control of the secretion of adenohypophysial hormones. Adv. metabol. Disorders **5**, 1 (1971).

HESS, W.R.: Das Zwischenhirn. Basel: Schwabe 1949, 1954.

LEVINE, R.: Endocrines and the central nervous system. Baltimore: Williams & Wilkins 1966.

LOCKE, W., SCHALLY, A.V. (eds.): The hypothalamus and pituitary in health and disease. Springfield, Ill.: Thomas 1972.

MARTINI, L., GANONG, W.F.: Neuroendocrinology. New York and London: Academic Press 1966.
— MEITES, J. (eds.): Neurochemical aspects of hypothalamic function. New York: Academic Press 1970.
— MOTTA, M., FRASCHINI, F. (eds.): The hypothalamus. New York and London: Academic Press 1970.
NALBANDOV, A.V. (Editor): Advances in neuroendocrinology. Urbana: Univ. of Illinois Press 1963.
REICHLIN, S.: Neuroendocrinology. New Engl. J. Med. **260**, 1182, 1246, 1296 (1963).
— Function of the hypothalamus. Amer. J. Med. **43**, 477 (1967).
SCHARRER, E., SCHARRER, B.: Neuroendocrinology. Columbia: Univ. Press 1963.
STUTINSKY, F.: Neurosecretion. Berlin-Heidelberg-New York: Springer 1967.
SZENTAGOTHAI, J., FLERKO, B., MESS, B., HALASZ, B.: Hypothalamic control of the anterior pituitary. An experimental-morphological study. 3rd (second english) edit. Budapest: Akadémiai Kiado 1968.

Embryology and Anatomy

BARGMANN, W.: Das Zwischenhirn-Hypophysensystem. Berlin-Göttingen-Heidelberg: Springer 1954.
— Neurohypophysis. Structure and function. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.
DIEPEN, R.: Der Hypothalamus. In: Handbuch der mikroskopischen Anatomie des Menschen. Hrsg. W. BARGMANN, Bd. IV/7. Berlin-Göttingen-Heidelberg: Springer 1962.
ENGELHARDT, FR.: Morphologische Grundlagen der Beziehungen zwischen Hypophyse und Hypothalamus. In: Handbuch der Neurochirurgie, Bd. I/2. Hrsg. H. OLIVECRONA und W. TÖNNIS. Berlin-Göttingen-Heidelberg: Springer 1968.
SCHARRER, E., SCHARRER, B.: Neurosekretion. In: W. VON MÖLLENDORF u. W. BARGMANN's Handbuch der mikroskopischen Anatomie des Menschen, Bd. VI/5. Berlin-Göttingen-Heidelberg: Springer 1954.
STARCK, D.: Embryologie. Stuttgart: Thieme 1965.

Biochemistry and Physiology

AKERT, K.: Physiologie und Pathophysiologie des Hypothalamus. In: SCHALTENBRAND und BAILEY, Einführung in die stereotaktischen Operationen. I. Stuttgart: Thieme 1959.
BABA, Y., MATSUO, H., SCHALLY, V.: Structure of the porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. Biochem. biophys. Res. Commun. **44**, 459 (1971).
BLANCO, S., SCHALCH, D.S., REICHLIN, S.: Control of Growth Hormonal-secretion by glucoreceptors in the hypothalamic-pituitary unit. Fed. Proc. **25**, 191 (1966).
BØLER, J., ENZMANN, F., FOLKERS, K., BOWERS C.Y., SCHALLY, A.V.: The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline-amide. Biochem. biophys. Res. Commun. **37**, 705 (1969).
BURGUS, R., DONN, TH.F., DESIDERIO, D.M., WARD, D.N., VALE, W., GUILLEMIN, R., FELIX, P.M., GILLESSEN, D., STUDER, R.O.: Biological activity of synthetic polypeptide derivatives related to the structure of hypothalamic TRF. Endocrinology **86**, 573 (1970).
DE GROOT, J.: Limbic and other neural pathways that regulate endocrine function. In: Neuroendocrinology, ed. by MARTINI and GANONG. New York and London: Academic Press 1966.
ENOCH, D.M., KERR, F.W.L.: Hypothalamic vasopressor and vesicopressor pathways. Arch. Neurol. (Chic.) **16**, 290 (1967).
FROHMAN, L.A.: Clinical neuropharmacology of hypothalamic releasing factors. New Engl. J. Med. **286**, 1391 (1972).
GESCHWIND, I.I.: Mechanism of action of releasing factors. In: Frontiers in neuroendocrinology, ed. GANONG, W.F., MARTINI, L. New York: Oxford University Press 1969.
GILLESSEN, D., FELIX, A.M., LERGIER, W., STUDER, R.O.: Synthese des Thyrotropin releasing-Hormons (TRH) (Schaf) und verwandter Peptide. Helv. chim. Acta **53**, 63 (1970).
HALL, R., AMOS, J., GARRY, R., BUXTON, R.L.: Thyroid-stimulating hormone response to synthetic thyrotrophin releasing hormone in men. Brit. med. J. **1970/II**, 274.
HAMMERSCHLAG, R., LEEMAN, S.E.: Induction of salivation by a bovine hypothalamic factor. Fed. Proc. **25**, 192 (1966).
HARRIS, G.W.: Humors and hormones. J. Endocr. **53**, II (1972).
HESS, W.R.: Die funktionelle Organisation des vegetativen Nervensystems. Basel: Benno Schwabe 1948.
MCCANN, S.M., PORTER, J.C.: Hypothalamic pituitary stimulating and inhibiting hormones. Physiol. Rev. **49**, 240 (1969).
MEITES, J.: Hypophysiotropic hormones of the hypothalamus: Assay and chemistry. Baltimore: Williams & Wilkins Co. 1970.
NAIR, R.M.G., BARRETT, J.F., BOWERS, C.Y., SCHALLY, A.V.: Structure of porcine thyrotropin releasing hormone. Biochemistry **9**, 1103 (1970).
— KASTIN, A.J., SCHALLY, A.V.: Isolation and structure of hypothalamic MSH release-inhibiting hormone. Biochem. biophys. Res. Commun. **43**, 1376 (1971).
NAUTA, W.J.H.: Central nervous organization and the endocrine motor system in NALBANDOV, A.V.: Advances in Neuroendocrinology. Urbana: Univ. Illinois Press 1963.
RAISMAN, G.: Neural connections of the hypothalamus. Brit. med. Bull. **22**, 197 (1966).
REICHLIN, S.: Functions of the median-eminence gland. New Engl. J. Med. **275**, 600 (1966).
ROTHBALLER, A.B.: Some endocrine manifestations of central nervous system disease. An approach to clinical neuroendocrinology. Bull. N.Y. Acad. Med. **4**, 257 (1966).
RUF, K., STEINER, F.A.: Steroid-sensitive single neurons in rat hypothalamus and midbrain: Identification by microelectrophoresis. Science **156**, 667 (1967).
SCHALLY, A.V., ARIMURA, A., BOWERS, C.Y., KASTIN, A.J., SAWANO, S., REDDING, T.W.: Hypothalamic neurohormones regulating anterior pituitary function. Recent Progr. Hormone Res. **24**, 497 (1968).
— BABA, V., BENNETT, C.D.: The amino acid sequence of a peptide with growth hormone-releasing activity isolated from porcine hypothalamus. J. biol. Chem. **246**, 6647 (1971).
SCHALTENBRAND, G., BAILEY, P.: Einführung in die stereotaktischen Operationen mit einem Atlas des menschlichen Gehirns. Stuttgart: Thieme 1959.
SCHARRER, E.: Principles of neuroendocrine integration. In: LEVINE, R., Endocrines and the central nervous system. Baltimore: Williams & Wilkins 1966.
— SCHARRER, B.: Hormones produced by neurosecretory cells. Recent Progr. Hormone Res. **10**, 183 (1954).
SPATZ, H.: Das Hypothalamus-Hypophysen-System in Hinsicht auf die zentrale Steuerung der Sexualfunktionen. Berlin-Göttingen-Heidelberg: Springer 1955.
STUDER, R., WERNER, I.: Thyrotropin-releasing factor (TRF). Schweiz. med. Wschr. **100**, 1086 (1970).
SZENTAGOTHAI, J., FLESKO, B., MESS, B., HALASZ, B.: Hypothalamic control of the anterior pituitary, 3rd ed. Budapest: Publ. House Hung. Acad. Sci. 1968.
VALE, W., BURGUS, R., DUNN, TH. F., GUILLEMIN, R.: Release of TSH by oral administration of synthetic peptide

derivatives with TRF activity. J. clin. Endocr. **30**, 148 (1970).

VEBER, D. F., BENNETT, C. D., MILKOWSKI, J. D., GAL, G., DENKEWALTER, R. G., HIRSCHMANN, R.: Synthesis of a proposed growth hormone releasing factor. Biochem. biophys. Res. Commun. **45**, 235 (1971).

YATES, F. E., RUSSELL, S. M., MARAN, J. W.: Brain-adenohypophysial communication in mammals. Ann. Rev. Physiol. **33**, 393 (1971).

Pathology and Clinics

ALBRIGHT, F., REIFENSTEIN, E. C.: The parathyroid glands and metabolic bone disease. Baltimore: Williams & Wilkins Company 1948.

BAUER, H. C.: Endocrine and other clinical manifestations of hypothalamic disease. A survey of 60 cases with autopsies. J. clin. Endocr. **14**, 13 (1954).

— Endocrine and metabolic conditions related to pathology in the hypothalamus: a review. J. nerv. ment. Dis. **128**, 323 (1959).

BETGHE, H., IRMSCHER, K., ZIMMERMANN, H.: Das Verhalten der Corticosteroide im Plasma während der Insulinhypoglykämie und unter Lysin-Vasopressin als Funktionsprüfung des Hypothalamus-Hypophysen-Nebennierenrinden-Systems. Acta endocr. (Kbh.) **55**, 622 (1967).

BIERICH, J. R., BRAUN, W.: Endokrine Störungen bei Zwischenhirnerkrankungen. Verh. dtsch. Ges. inn. Med. **71**, 282 (1965).

COURVOISIER, B., FASEL, J., VERNET, A., BERTHOUD, E.: Tuberculose atypique avec syndrome hypothalamo-hypophysaire progressif aboutissant à un panhypopituitarisme. Schweiz. med. Wschr. **90**, 870 (1960).

EIK-NES, K., CLARK, L. D.: Diurnal variation of plasma 17-hydroxycorticosteroids in subjects suffering from severe brain damage. J. clin. Endocr. **18**, 764 (1958).

HALL, R., WARRICK, CH.: Hypersecretion of hypothalamic releasing hormones: A possible explanation of the endocrine manifestations of polyostotic fibrous dysplasia (Albright's syndrome). Lancet **1972 I**, 1313.

HEDINGER, CHR., HÜRZELER, D.: Hypopituitarismus bei Dystopie des Hypophysenhinterlappens. Acta endocr. (Kbh.) **14**, 170 (1953).

HÖKFELT, B., LUFT, R.: The effect of suprasellar tumours on the regulation of adrenocortical function. Acta endocr. (Kbh.) **32**, 177 (1959).

KAHANA, L., KAHANA, S., MCPHERSON, H. T.: Endocrine manifestations of intracranial extrasellar lesions. In: BAJUSZ, Clinical neuroendocrinology. Basel-New York: Karger 1967.

— LEBOVITZ, H., LUSK, W., MCPHERSON, H. T., DAVIDSON, E. T., OPPENHEIMER, J. H., ENGEL, F. L., WOODHALL, B., ODOM, G.: Endocrine manifestations of intracranial extrasellar lesions. J. clin. Endocr. **22**, 304 (1962).

KRAYENBÜHL, H., ZOLLINGER, H. U.: Malignes metastasierendes Pinealocytom mit dem klinischen Bild der Dystrophia adiposo-genitalis. Schweiz. Arch. Neurol. **51**, 77 (1943).

KRIEGER, D. T., KRIEGER, H. P.: Effects of organic brain lesions on adrenal cortical activity. In: LEVINE, R., Endocrines and the central nervous system. Baltimore: Williams & Wilkins 1966.

LANDON, J., GREENWOOD, F. C., STAMP, T. C. G., WYNN, V.: The plasma sugar, free fatty acid, cortisol and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function. II. In Patients with hypothalamic or pituitary dysfunction or anorexia nervosa. J. clin. Invest. **45**, 437 (1966).

LANGE-COSACK, H.: Zur Frage der hypothalamischen Pubertas praecox. In: Zentrale Steuerung der Sexualfunktionen. Berlin-Heidelberg-New York: Springer 1966.

LINQUETTE, M., LAINE, E., FOSSATI, P., LEFEBVRE, J.: Pinéalome ectopique avec insuffisance hypophysaire antérieure, adipsie et hypernatrémie neurogene. Ann. Endocr. (Paris) **28**, 39 (1967).

LIPSETT, M. B., DREIFUSS, F. E., THOMAS, L. B.: Hypothalamic syndrome following varicella. Amer. J. Med. **32**, 471 (1962).

MARTIN, L. G., MARTUL, P., CONNOR, T. B., WISWELL, J. G.: Hypothalamic origin of idopathic hypopituitarism. Metabolism **21**, 143 (1972).

MORGAN, T., COUPLAND, W. G., VANDERFIELD, G. K., CHURCH, D.: Hypothalamic-pituitary sarcoidosis. Aust. Ann. Med. **14**, 250 (1965).

NOWAKOWSKI, H.: Über die endokrine Symptomatik bei Erkrankungen des Hypothalamus. Verh. dtsch. Ges. inn. Med. **61**, 49 (1955).

OPPENHEIMER, J. H., FISHER, L. V., JAILER, J. W.: Disturbance of the pituitary-adrenal interrelationship in diseases of the central nervous system. J. clin. Endocr. **21**, 1023 (1961).

ORTHNER, H.: Pathologische Anatomie und Physiologie der hypophysär-hypothalamischen Krankheiten. In: Handbuch der speziellen pathologischen Anatomie und Histologie, O. LUBARSCH, F. HENKE, R. RÖSSLE, Bd. XIII, Nervensystem, Teil 5, S. 543—939. Berlin-Göttingen-Heidelberg: Springer 1955.

ROTHBALLER, A. B.: Some endocrine manifestations of central nervous system disease. Bull. N.Y. Acad. Med. **42**, 257 (1966).

WOLFF, S. M., ADLER, R. C., BUSKIRK, E. R., THOMPSON, R. H.: A syndrome of periodic hypothalamic discharge. Amer. J. Med. **36**, 956 (1964).

WYK, J. J. VAN, DUGGER, G. S., NEWSOME, J. F., THOMAS, P. Z.: The effect of pituitary stalk section on the adrenal function of women with cancer of the breast. J. clin. Endocr. **20**, 157 (1960).

Added in Proofs:

BRAZEAU, P., VALE, W., BURGUS, R., LING, N., BUTCHER, M., RIVIER, J., GUILLEMIN, R.: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science **179**, 77 (1973).

GUAL, C., ROSEMBERG, E. (eds.): Hypothalamic hypophysiotropic hormones; physiological and clinical studies. Excerpta med. (Amst.) (1973).

SCHALLY, A. V., ARIMURA, A., KASTIN, A. J.: Hypothalamic regulatory hormones. Science **179**, 341 (1973).

WILBER, J. F.: Thyrotropin releasing hormone: secretion and actions. Ann. Rev. Med. **24**, 353 (1973).

III. The Hypothalamo-Neurohypophyseal System

A. LABHART

With Contributions by

G. TÖNDURY and G. KISTLER

A. Historical Dates

1794 J. P. FRANK distinguished diabetes insipidus from diabetes mellitus.
1895 OLLIVER and SCHÄFER produced an extract from the pituitary which caused a rise in blood pressure.
1910 A. FRANK associated diabetes insipidus with a hypofunction of the posterior pituitary.
1913 CAMUS and ROUSSY produced diabetes insipidus in dogs by hypothalamic lesions, without injury to the pituitary.
1913 FARMI and VON DEN VELDEN established the therapeutic effect of extracts of the posterior pituitary in diabetes insipidus.
1948 VERNEY showed that the activity of the hypothalamic-neurohypophyseal system was regulated by the osmotic concentration of the blood in the hypothalamus.
1953/55 DU VIGNEAUD isolated and synthesized vasopressin and oxytocin.

B. Embryology, Gross Anatomy and Histology

G. TÖNDURY and G. KISTLER

1. The Neurohypophysis

The hypophysis has two major subdivisions which differ fundamentally in embryology, morphology, and function. The *adenohypophysis* is derived from a dorsal outpocketing of the ectodermal buccopharyngeal region. It demonstrates all the characteristics of an endocrine gland. Some aspects of its development and anatomy are discussed in Chap. V, p. 77. The *neurohypophysis* originates from the diencephalon. In the 7–8 mm-long human embryo, the neurohypophyseal anlage appears first as a thickening of the diencephalic floor. From this, a compact epithelial process grows downward behind the anlage of the adenohypophysis and develops into infundibulum and neural lobe.

Gross Anatomy. The fully developed neurohypophysis (see also p. 78) has three subdivisions: 1. The *infundibulum* (neural stalk, pars proximalis of the neurohypophysis) extends from the floor of the diencephalon down to the diaphragma sellae of the sphenoid bone. It forms the *neuro*hypophyseal portion of the hypophyseal stalk. Its boundary towards the tuber cinereum is given by the hypothalamo-hypophyseal sulcus (sulcus tuberoinfundibularis). At the root of the infundibulum, the third ventricle evaginates to form the infundibular recess (Fig. 1). The *median eminence*, a funnel-shaped extension of the tuber cinereum, must morphologically and functionally be considered as part of the infundibulum. 2. The *infundibular stalk* is the border area between infundibulum and neural (posterior) lobe. It contacts with both the pars tuberalis and the pars intermedia of the adeno-

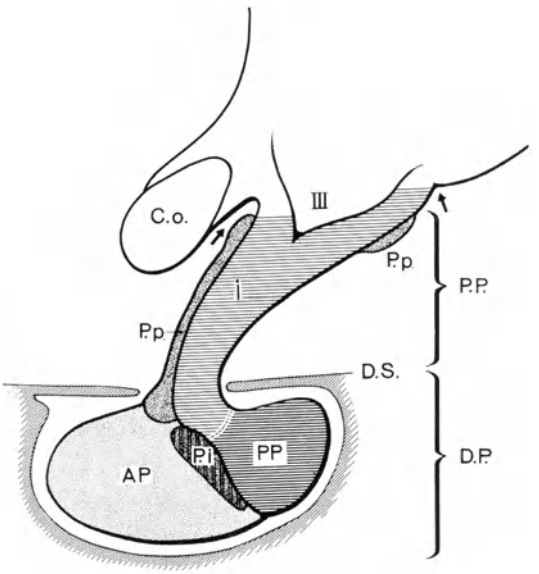


Fig. 1. Part of the pituitary of the adult man. Scheme with reference to ENGELHARDT (1968). *P.P.* proximal suprasellar pituitary; *D.P.* distal, intrasellar pituitary; *I* Infundibulum; *P.p.* pars infundibularis adenohypophysea; *D.S.* diaphragma sellae; *AP* anterior pituitary; *P.i.* pars intermedia (intermediate lobe); *PP* posterior pituitary; *C.o.* chiasma opticum; *III* third ventricle with recessus infundibuli. The darts indicate the sulcus hypothalamo-hypophyseus

hypophysis. The *infundibular process* (3) (neural lobe, pars distalis of the neurohypophysis) is lodged within the sella turcica of the sphenoid bone, adjacent to the pars intermedia of the adenohypophysis.

Blood Supply. The hypophysis is supplied by the left and right *superior* hypophyseal arteries which arise from the internal carotids near the origin of the ophthalmic arteries. Each superior hypophyseal artery divides into an anterior and a posterior branch (anterior and posterior superior hypophyseal artery, respectively). In the median eminence, the *anterior* branches form characteristic capillary loops which drain into the hypophyseal portal vessels. These in turn supply the sinusoidal capillary network in the adenohypophysis (see also p. 82). The anterior branches also supply the upper parts of the pars infundibularis of the adenohypophysis and, in addition, give rise to a small trabecular artery which passes directly into the anterior lobe where it anastomoses with the inferior hypophyseal artery. The *posterior* branches break up into a capillary network within the infundibulum. The region of the tuber cinereum receives additional blood from branches of the posterior communicating arteries. The neural lobe and the lower portions of both the infundibulum and the pars infundibularis of the adenohypophysis are supplied by the *inferior* hypophyseal arteries. These are also branches of the internal carotids and anastomose with the trabecular arteries. The *venous blood* is conveyed by capsular veins of the hypophysis into the anterior and posterior intercavernous sinuses which drain into the cavernous sinus. Thus, the hypophysis is surrounded by large blood channels anteriorly, posteriorly, and bilaterally (circular sinus).

Intra- and suprasellar portions of the hypophysis are in close proximity to vital brain structures and blood vessels. Through the foramen hypophyseale of the diaphragma sellae, the sella turcica of the sphenoid bone opens into the middle cranial fossa. The lateral wall of the dura mater lining the sella is also the middle wall of the cavernous sinus. The hypophyseal stalk, which connects the intrasellar portions of the hypophysis with the hypothalamus, is situated immediately beneath the optic chiasma. Although morphological variations of the chiasma are frequent, its close spatial relationship to the hypophyseal stalk is a constant feature. Tumors of the adenohypophysis can damage the decussating nerve fibers in the chiasma and lead to bitemporal hemianopsia. The proximal part of the infundibulum of the neurohypophysis encloses the infundibular recess of the third ventricle (Fig. 2) and thus gains close connections with the ventricular system of the brain. In the region of the perinfundibular cistern, the hypophyseal stalk is also surrounded by cerebrospinal fluid. In addition, the infundibulum lies in the center of the arterial circle of Willis and in close proximity to the small but numerous arteries of the olfactory area. This area joins the infundibulum rostrally and laterally. Finally, the tuber cinereum and the two mamillary bodies are also situated close to the infundibulum.

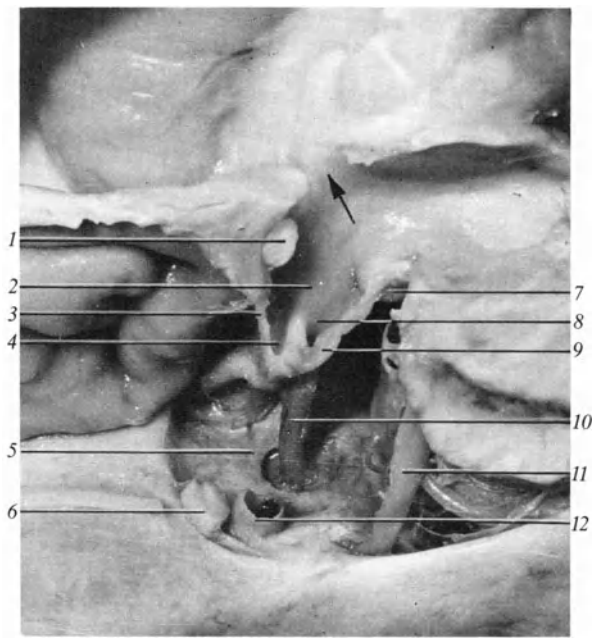


Fig. 2. Topography of the hypophyseal stalk in the adult. *1* Commissura anterior; *2* 3rd ventricle; *3* Lamina terminalis; *4* Recessus chiasmatis ventriculi tertii; *5* diaphragma sellae with foramen hypophyseos; *6* Fasciculus opticus; *7* Corpus mammillare; *8* Recessus infundibuli; *9* Tuber cinereum; *10* Infundibulum; *11* Nervus oculomotorius; *12* Arteria carotis interna. The dart indicates the foramen interventriculare Monroi

Histology. In routine histological sections, the neurohypophysis appears as a mass composed of innumerable ill-defined fibers, among which a rather heterogeneous population of cells is distributed. In sections stained with silver salts, a fine network of argyrophilic fibers is found to surround the blood vessels branching within the organ. Like the adenohypophysis, the neurohypophysis is enclosed by a relatively thick connective tissue capsule. From this capsule, a thin septum rises between the anterior and posterior lobe (pars intermedia) towards

the brain. This boundary may in parts be erased by a penetration of basophilic cells from the pars intermedia of the adenohypophysis into the posterior lobe.

The neurohypophysis contains two major elements. The bulk of its substance is made up of about 100000 *unmyelinated nerve fibers*, whose cell bodies are located in the hypothalamus. Distributed among these fibers are the *pituicytes*, which vary greatly in shape and size. Due to their structural relation to the axons, these cells must be considered as a special form of the neuroglia. They commonly contain various amounts of pigment granules. A broad perivascular space bounded on all sides by a basal lamina separates the nerve fiber endings and the pituicyte processes from the network of blood capillaries with fenestrated endothelia. Light microscopy reveals the characteristic strongly positive PAS-reaction of this perivascular area, which indicates the presence of large amounts of mucopolysaccharides. In the electron microscope, finger-shaped ramifications from this space can often be traced for a good distance between the axons and the pituicytes.

2. The Hypothalamo-Neurohypophyseal Connections

The axons comprising the major part of the neurohypophyseal mass collectively form the hypothalamo-neurohypophyseal tract. Its cell bodies (perikarya) are located mainly within the supraoptic and paraventricular nuclei, whereas other hypothalamic nuclear masses apparently contribute only a few nerve fibers. The axons pass via the infundibulum and the infundibular stalk to reach the posterior lobe where they terminate in intimate contact with the capillaries. When special staining methods (e.g. aldehydefuchsine) are used, variously sized, intensively staining masses can be recognized in both the cell bodies of the hypothalamic nuclei and in their axons. These are the so-called *Herring bodies*. In the electron microscope, these bodies have been found to be locally dilated portions of the axons where granules are densely packed with mitochondria and other cell organelles. The granules are thought to contain the hormones synthesized on the granular endoplasmic reticulum (Nissl substance) and bound to a carrier protein in the Golgi complex. They are carried along the entire length of the axons into the posterior lobe. In addition to these neurosecretory granules with a diameter of about 100 to 200 nm, the nerve fiber endings also contain smaller vesicles of about 40 nm diameter. Such vesicles are normally found in synapses elsewhere in the nervous system. Their function at this site is still a matter of dispute.

The two hormones formed in the supraoptic and paraventricular nuclei are oxytocin and vasopressin. Since no neurosecretory granules have been demonstrated within the extensive perivascular space separating the capillary endothelia from the pituicytes and the nerve fiber endings, it must be assumed that the granules are dissolved while still within the axon terminals and that their content is emptied in molecular form into the cytoplasmic ground substance. From there, after passing through the axonal cell membrane, the hormones reach the perivascular space rich in mucopolysaccharides and mucoproteins. One might speculate that this material acts less as a barrier and more as a distribution system ensuring unimpeded and rapid transfer of the hormones into the capillary system. The function of the pituicytes in this process has not yet been clarified. Increasing evidence indicates, however, that these cells do not only have a supportive function, but that they are largely involved in the metabolism of the neurosecretory cells and their axons.

C. Biochemistry, Transport, Inactivation, and Excretion

The hypothalamic-neurohypophyseal system of the human and of other mammals produces two hormones: vasopressin and oxytocin. They are closely related chemically and their actions overlap to a slight extent.

Both hormones are octapeptides, and both were isolated and synthesized by DU VIGNEAU. Oxytocin and vasopressin were the first hormones to be synthetically prepared. These synthesized hormones show no chemical, physical or biological differences from the naturally isolated hormones. The name vasopressin originated from the first, probably unphysiological effect of the hormone to be discovered, namely the blood pressure-raising effect. However, the terms adiuretin or antidiuretic hormone have not managed to prevail. Neurohypophyseal extracts with predominantly antidiuretic and pressor effects are termed as pitressin. Extracts with an oxytocin action are designated as pituitrin or pitocin.

In mammals there are two vasopressins. The human and most other mammals form arginine-vasopressin (LIGHT, 1958). The pig and its relations produce lysin-vasopressin. The remaining vertebrates form vasotocin instead of vasopressin, and certain osteichthyes produce

Table 1. Structural formula of the 7 neurohypophyseal hormones presently separated in nature using the usual abbreviations for the amino-acid residues (according to BERDE, 1964; HELLER, 1966; BOISSONAS, 1968)

Name	Formula	Molecular-weight
	1 2 3 4 5 6 7 8 9	
Oxytocin	H—Cys—Tyr—Ileu—Glu(NH_2)—Asp(NH_2)—Cys—Pro—Leu—Gly—NH_2	1007
Lysin-Vasopressin	H—Cys—Tyr—Phe—Glu(NH_2)—Asp(NH_2)—Cys—Pro—Lys—Gly—NH_2	1056
Arginine-Vasopressin	H—Cys—Tyr—Phe—Glu(NH_2)—Asp(NH_2)—Cys—Pro—Arg—Gly—NH_2	1084
Vasotocin	H—Cys—Tyr—Ileu—Glu(NH_2)—Asp(NH_2)—Cys—Pro—Arg—Gly—NH_2	
Ichthyotocin (Isotocin)	H—Cys—Tyr—Ileu—Ser—Asp(NH_2)—Cys—Pro—Ileu—Gly—NH_2	
Mesotocin	H—Cys—Tyr—Ileu—Glu(NH_2)—Asp(NH_2)—Cys—Pro—Ileu—Gly(NH_2)	
Glumitocin	H—Cys—Tyr—Ileu—Ser—Asp(NH_2)—Cys—Pro—Glu(NH_2)—Gly(NH_2)	

Table 2. Phylogenetic distribution and quantitative pharmacological characterization, in international units per milligram, of 5 neurohypophyseal hormones. The five pharmacological methods used here are usually employed for the characterization of the "oxytocin-like", or "vasopressin-like" properties of the substance. (After BERDE, 1964)

Neurohypophyseal hormones	Oxytocin-like activities (IU/mg)			Vasopressin-like activities (IU/mg)		Phylogenetic distribution
	Uterus (rat *in vitro*)	Blood pressure (cock)	Milk glands (rabbit)	Blood pressure (rat)	Antidiuresis (rat)	
Arginine-Vasopressin	16± 4	60± 6	65± 8	400±40	400±40	Many mammals
Lysin-Vasopressin	5± 0.5	40± 5	60±10	270±20	250	Pig, hippopotamus, pekari
Oxytocin	450±30	450±30	450±30	5± 1	5± 1	Many vertebrates
Vasotocin	115±15	285±40	210	245±15	250±35	Vertebrates with exception of mammals
Ichthyotocin, Isotocin	150±12	320±15	300±15	0.06± 0.01	0.18± 0.03	Some osseous fishes (osteichthyes)

ichthyotocin or isotocin (BERDE, 1964; SAWYER, 1966; ACHER, 1966; WALTER, 1967). See SAWYER (1967), VLIEGENTHART (1967), and ACHER (1969) for the phylogenetic relation of the hormones of the posterior lobe and their function in nonmammals (Table 1, 2).

Oxytocin and arginine-vasopression differ in only two amino-acid residues. In mammals, oxytocin predominantly causes the uterus to contract and milk to be ejected. On the other hand, vasopressin has mainly an antidiuretic effect, and in higher concentrations pressor properties. The actions overlap, and this would appear to be physiologically rational to some extent, as, for example, antidiuresis in lactation.

Vasopressin and oxytocin are stored in the neurohypophysis and bound to specific carrier proteins, neurophysin I and neurophysin II, in granules visible by electron microscopy. Neurophysin I binds more oxytocin; neurophysin II has a selective affinity for vasopressin. Vasopressin and oxytocin are usually secreted together with the neurophysins into the blood, and free hormones may also be released. Calcium ions shift during the process of release into the cells; at a certain concentration they inhibit binding to the neurophysins (GINSBURG, 1966).

It is still uncertain how much vasopressin and oxytocin circulate in the free form and to

what extent they are bound in the plasma. Neurophysins circulate in a concentration of 1.3 to 40 ng/ml in the blood, according to the state of hydration and diuresis. Neurophysins are higher in women than in men, and are elevated in pregnancy and in renal insufficiency (LEGROS, 1972; SACHS, 1969). Vasopressin is evenly distributed within the plasma volume and only a small percentage is transferred to the extracellular fluid. The half-life in the human, estimated using vasopressin labeled with tritium and ^{131}I, is extremely short, approximately 4 minutes (SILVER, 1961). However, when estimated from infusion studies, it is reported to be 16–17 min (CZACZKES, 1964). The majority of the vasopressin is inactivated in the liver and kidneys (LAUSON, 1961). A relation between receptor-effect and inactivation is under discussion (DICKER, 1961). Only 5–20% is excreted through the kidneys. The greater part of the vasopressin excreted in the urine is not dialyzable (THORN, 1959). The nature of the carrier protein in urine is unknown.

The concentration of vasopressin in human plasma lies below or just at the lower limit of biological assessment, even in blood from the internal jugular vein and during extreme dehydration. Infusion methods yield concentrations of 1–5 μU/ml = 0.01 mμg/ml (LAUSON, 1967; SCHRÖDER, 1959; SHARE, 1967). Secretion is estimated to be 7.5–50 mU/h, and the total clearance has been calculated to be 150 ml/min (LAUSON, 1967). These estimations by biological assays are roughly confirmed by the difficult radioimmunological method of determination which has recently become available and which allows measurement of concentrations of vasopressin down to 1 pg/ml. In healthy persons, the vasopressin concentration is found to be between 1 and 10 μμg/ml according to the state of hydration. The concentration can rise to 26 μμg/l in nephrogenic diabetes insipidus (ROBERTSON, 1970).

The clearence of vasopressin is elevated in central and nephrogenic diabetes insipidus. Despite the low molecular weight, many animals and also a few humans can form circulating antibodies against lysin-vasopressin or arginine-vasopressin (ROTH, 1966).

Oxytocin circulates bound to beta-globulins in a volume corresponding to approximately 40% of the body fluid. The half-life in the pregnant woman is approximately 3 min. Oxytocin is also inactivated by the liver, the kidney and the mammae during lactation. In addition, during pregnancy in primates, oxytocinase appears in the plasma which also disintegrates vasopressin. Its origin and physiological significance are, however, still unknown.

The synthesis of these polypeptide hormones permits the production of derivatives with altered activity, which can be pharmacologically useful. Over 200 analogues have been synthesized and examined (WALTER, 1967), revealing certain correlations between structure and effect (BOISSONAS, 1961). The substitutions in positions 3 and 8 are of special interest. Inhibitors can also be produced, and conclusions about the mode of action can be drawn from the molecular structure (WALTER, 1967). The synthesized hormones are sometimes more effective per unit weight than the natural hormones. Thus, the antidiuretic effect of desamino 1-arginine-vasopressin is three times greater than that of the naturally occurring hormone (BERDE, 1964). See p. 61 for information on DDVAP. 2-Phenylalanine 8-lysine-vasopressin, which has only a slight antidiuretic action but a definite pressor effect (GUHL, 1960) has been introduced into the treatment for hemorrhage of esophageal varices since it produces an arteriolar constriction in the splanchnic area with a fall in portal venous pressure (TSAKIRIS, 1964). Ornithin^{8}-vasopressin has a yet more favorable pressor/antidiuretic ratio and is used for local hemostasis (BERDE, 1964). See BERDE (1966) for the synthetic analogues and homologues. See WOOLEY (1956) for the specificity of the peptide hormones (oxytocin effect of angiotensin, vasopressin action of pepsitensin).

D. Physiology

1. Place of Formation of Vasopressin and Oxytocin

Vasopressin and oxytocin are formed in different neurons of the hypothalamic nuclei and reach the neurohypophysis by way of the nerve tracts (neurosecretion) (BARGMANN, 1954; SCHARRER, 1954; LEDERIS, 1962).

The ganglion cells of the supra-optical and paraventricular nuclei, the supra-optico-hypophyseal tract, and the neurohypophysis form a unit, which when interrupted at any point leads to functional failure of the system and thereby to diabetes insipidus. Usually the degree of the functional disturbance is proportional to the extent of the lesion. Removal of the neurohypophysis or division of the supra-optico-hypophyseal tract leads to the degeneration of a number of neurons, which increases with increasing proximity of the lesion to the ganglion cells. The neurosecretory neuron has a special place between the cells. Apart from the elements of the ordinary nerve cell, such as dendrites,

axon, neurofibrils and myelin sheath, it also contains granules of various sizes in the ganglion cell itself and along the axis cylinder. These granules can be selectively stained by GOMORI's method. This Gomori-positive or neurosecretory substance becomes more dense distally and is found in particular abundance in the neurohypophysis. Valuable support for the opinion that this substance is related to the hormonal activity of the system is obtained from the fact that the quantity of these neurosecretory granules is dependent on the functional state of the system and that the content of antidiuretic hormone of the areas surrounding the hypothalamus and neurohypophysis corresponds quantitatively to the amount of neurosecretory substance in these areas. The ganglion cells increase in size during thirst and after osmotic stress (functional nuclear edema). At the same time, there is a decrease in the neurosecretory substance as well as in the antidiuretic hormone content in the distal regions, especially in the neurohypophysis. After a water load, the neurohypophysis again becomes filled with neurosecretory substance.

This neurosecretory substance is definitely formed in the ganglion cells of the supra-optical nucleus and possibly in the paraventricular nucleus as well. Experiments show that oxytocin is formed in the paraventricular nucleus, whereas vasopressin is produced in the supra-optical nucleus (OLIVECRONA, 1957). These substances then migrate along the supra-optico-hypophyseal tract, and 80% is stored in the neurohypophysis as subcellular granular elements of 100–300 mμm diameter. When the pituitary stalk is interrupted, the neurosecretory substance stagnates in the proximal end of the stump, whereas the distal end discharges the substance. In tissue cultures of the ganglion cells of the dog's supra-optical nucleus, a movement distally of granulated elements could be demonstrated with rapid motion pictures. Finally, tests with substances labeled with ^{32}P showed migration in the direction distal to the ganglion cells. The velocity of the transport varies with the degree of the osmotic stress. The neurosecretory substance seems to move with the plasmatic flow of the axon.

No antidiuretic hormone has been demonstrated in other hypothalamic nuclei. The neurosecretory substance is not identical to the hormones of the hypothalamic-neurohypophyseal system. As far as can be determined histochemically, it appears to be a lipoglycoprotein which, like a carrier protein, combines with the hormonally active polypeptides.

In mammals, the ganglion cells of the supra-optical and paraventricular nuclei are the only cells so far known to produce a carrier substance of this kind with hormonal activity.

2. Release

The release of oxycotin and vasopressin results from numerous nervous, chemical, and physical stimuli. Pharmacological, physiological and pathological stimuli can be differentiated among the indirect stimuli.

Drugs, in particular acetylcholine, nicotine, and many analgesics, hypnotics and anesthetics, such as morphine, ether, and barbiturates, lead to the release of vasopressin. Intravenous administration of 0.5–1.0 mg nicotine (3 mg in smokers) causes the release of vasopressin when the hypothalamo-neurohypophyseal system is intact (nicotine test, see p. 59 f.). Although acetylcholine and adrenaline in small doses inhibit the release of vasopressin, adrenaline in large doses has the opposite effect.

A rise in plasma osmotic pressure is the specific physiologic stimulus causing a release of vasopressin. The act of suckling results in the release of oxytocin. Vasopressin and oxytocin can be released together, or a specific stimulus may cause one or the other to be secreted in predominance (ACHER, 1958; HELLER, 1966). However, overlapping occurs. Thus, suckling causes a simultaneous inhibition of diuresis, and the administration of hypertonic saline leads to the ejection of milk and to contraction of the uterus. In mammals, including the human, stimulation of the genital organs and coitus also result in the release of oxytocin and vasopressin. In the nursing mother milk is released, whereas in the male and other females diuresis is retarded (HARRIS, 1953; FRIBERG, 1953).

Changes of 1% in the osmolality (see p. 46 f.) of the plasma are sufficient to induce or inhibit vasopressin secretion. The chemical nature of the substances dissolved in the plasma is, however, unimportant, providing they are not prevented from exerting an effect on the end organ through excessive diffusibility (e.g. glucose, urea). VERNEY showed in his precise experiments in the dog that the electrolytes and the osmotic pressure of the total blood have little effect on the release of vasopressin. On the other hand, an 8% rise in the chloride content of the internal carotid artery, corresponding to an increase of 2% in the osmotic pressure, induces the neurohypophysis to release 1 micro unit of vasopressin per second = 3.6 mU/h), diminishing the urinary output to less than 1 $cm^3/m^2/min$. There must, therefore, be osmoreceptors in the area supplied by the internal carotid arteries. Presumably, these receptors

cause the neurosecretory cells of the hypothalamo-neurohypophyseal system to release vasopressin into the blood.

The bubbles in the supra-optical and paraventricular nuclei, considered by VERNEY to be osmo-receptors, are really vacuoles of the ganglion cells with a laterally placed signet ring-shaped nucleus. They are only found in a few species and vary in size and number according to the osmotic stress. They are, therefore, to be considered as a product and not as a controlling organ. The ganglion cells themselves must possess osmo-receptor properties since their action currents vary according to the osmotic stress. The osmo-receptors are situated in the region supplied by the internal carotid artery, but the localization has not yet been quite successfully determined (JEWELL, 1957).

On the other hand, various localizations of the release regulation have been established for the nervous, pharmacological and osmotic stimuli (DINGMAN, 1957).

The release of vasopressin is not controlled by the absolute osmotic pressure but rather by the gradient between the extra- and intracellular osmotic pressures. The difference between the two depends mainly upon the extracellular sodium concentration. On average, the release of vasopressin in man begins at an osmolality of over 285 mOsm/kg (MOSES, 1967).

The release or inhibition of vasopressin probably results immediately from perceptible variations in the osmotic pressure. Cortisol increases the stimulus threshold for the release of vasopressin, even when nicotine (DINGMAN, 1957, 1958) or osmotic stimuli are employed (AUBRY, 1965). Increase in blood volume (see beneath) also increases the osmotic stimulus threshold (MOSES, 1967).

In addition to osmoreceptors, volume receptors and baroreceptors, probably in the left atrium, the carotid sinus and in the aortic arch, are supposed to control diuresis through vasopressin secretion (GAUER, 1963; LEAF, 1952; HARE, 1967). Osmotically induced diuresis is inhibited by a slight decrease in plasma volume. Volume regulation dominates the osmoregulation (ARNDT, 1965). The "effective circulating blood volume" appears to be the critical factor rather than the extracellular fluid volume (SHARE, 1962). Vasopressin and oxytocin are liberated from the granules and pass through the cell membranes to reach the blood. Calcium ions appear to have an effect on the liberation of both vasopressin and oxytocin from their carrier substances (THORN, 1965). Only the 20% of vasopressin stored in the tissue can be released on a specific stimulus. Emotions, pain, vomiting, and loss of blood are some of the stimuli which can cause a release of vasopressin. Conversely, antidiuresis can be interrupted by hypnosis or certain reflexes (HOFER, 1963; HULET, 1963). The very variable enhancing or inhibitory stimuli must be received and integrated by a hypothalamic center. This converts them into an impulse for the release of vasopressin (PITTS, 1968).

There are only hypothetical views about the mechanism of the hormonal release from the posterior pituitary lobe into the blood. These include neural impulses, alterations in electrical potentials with electrolytic displacement, in particular the entrance of calcium ions into the cells, or permeability changes (HELLER, 1966). In addition to the immediately available portion of vasopressin, the posterior lobe contains more vasopressin which is only slowly released (SACHS, 1967, 1969). Temporal observations would make a neurohumoral transmission appear improbable, but support the concept of transmission by neural impulses (so-called stimulation-secretion-coupling, SACHS, 1967). These impulses are transmitted either by the neurofibrils of the secretory neurons or by the numerous neighboring unmyelinated nerve fibers.

3. Action of the Neurohypophyseal Hormones

a) Oxytocin

Oxytocin exerts a constricting effect on the uterine muscle. Estrogens increase the response of the uterus to oxytocin, whereas progesterone inhibits it. During pregnancy, the sensitivity of the uterus to oxytocin is greatly reduced. However, during delivery and immediately after, the uterus is particularly sensitive to oxytocin. There are many data supporting the physiological effect of oxytocin during the stages of labor (see p. 692). Further clarification will only result from the radioimmunological assay techniques recently introduced.

In addition to the effect noted above, oxytocin causes contraction of the myoepithelial elements of the excretory ducts of the mammary glands, resulting in milk ejection. In the rat and dog, oxytocin also has a diuretic effect by virtue of enhanced electrolyte excretion. This is not so in man. The effect of oxytocin on the release of prolactin is questionable, and it is not likely that oxytocin has any physiological function in the male genital system (FITZPATRICK, 1966). Finally, oxytocin and its analogues, such as vasopressin, in high doses have an insulin-like effect on glucose metabolism *in vitro* upon rat adipose tissue.

b) Vasopressin

α) Regulation of Water Intake and Diuresis

The hypothalamus, neurohypophysis and kidneys constitute a system for maintaining constant osmotic pressure or constant osmolality (see p. 46f.) of the extra- and intracellular body fluids. Like other regulatory systems, it is concerned with homeostasis. It generally succeeds in maintaining a constant internal environment, which was recognized by CLAUDE BERNARD as being essential to life.

Water intake and loss are controlled by the hypothalamus, which registers and integrates osmolality, volume of body fluids and other stimuli (Table 3).

The water intake is controlled predominantly by the thirst center. This center is situated in the ventro-medial hypothalamus, between the descending column of the fornix and the mamillo-thalamic tract, caudal to and partly overlapping the osmoreceptors controlling vasopressin secretion (ANDERSSON, 1955). Stimulation of this area with very small amounts of hypertonic saline or with an electric current results in compulsive water drinking of up to 40% of the body weight. Polyuria follows only after a latent period of several hours. Destruction of this hypothalamic area causes complete or partial adipsia. The specific stimulus inducing thirst has not been definitely identified; however, extra- and intracellular hyperosmotic dehydration appear to be predisposing conditions. An increase in the osmolality alone is not sufficient, and alterations in volume seem to be involved. It is not known whether the osmoreceptors for the release of vasopressin are also concerned with the induction of thirst, functioning through different nerve tracts, or if other receptors, partly overlapping with the osmoreceptors for vasopressin, are also present (ANDERSSON, 1957; FITZSIMMONS, 1972). A dry buccal mucosa is only an additional factor in inducing thirst. The water loss is controlled by the kidneys under the influence of the release of hypothalamic vasopressin.

The kidneys play a critical role among all organs capable of excreting water since the lungs, stomach and skin excrete water with no concern for the hydration state of the body. Apparently, these latter organs are so very much concerned with their own specialized function that they neglect the economy of the water equilibrium in preference to their own functional maintenance (lungs: CO_2 excretion; stomach: HCl production; skin: temperature regulation). In mammals, the kidney is the only secretory and excretory organ capable of delivering a hypertonic excretion, the urine. It is therefore well suited to the function of conserving water.

The body can tolerate wide ranges of variations in the amount of extracellular water. There is also a relatively wide margin of tolerance to the absolute quantities of the substances dissolved in the body fluid. The ratio of water to the dissolved substances, however, must be kept within relatively narrow limits. This is normally 300 mOsm/kg H_2O, and is dependent predominantly on the sodium concentration in the plasma. The Na concentration can vary between 120–160 mEq/l.

The unit osmol is used for measuring the number or the mass of the osmotically active molecules in an aqueous solution. It corresponds to a number or mass (according to the definition) of 6.023×10^{23} osmotically active particles, and is equal to a mol in undissociated substances. As long as the substance dissociates in solution, the number of osmotically active particles increases by a factor identical with the number of ions into which the molecule dissociates.

The osmotic pressure (as well as many other physical properties) of a solution, is a linear function of the relation between the number of osmotically active molecules and the number of molecules of the dissolving agent. This ratio or this quotient of the number of osmotically active

Table 3. Control of diuresis through the hypothalamic-neurohypophyseal-renal system (↓ decrease, ↑ increase) (TALBOT, 1952)

	Hemoconcentration	Hemodilution
Blood	Osmolality ↑	Osmolality ↓
	↓	↓
Hypothalamus	Osmoreceptors	Osmoreceptors
	↓	↓
Neurohypophysis	Release of vasopressin	Inhibition of vasopressin secretion
	↓	↓
Blood	Vasopressin ↑	Vasopressin ↓
	↓	↓
Kidneys	Distal water reabsorption ↑	Distal water reabsorption ↓
	↓	↓
Urine	Osmolality ↑	Osmolality ↓
	Specific gravity ↑	Specific gravity ↓

molecules in osmols to the amount of solvent in kg is the osmolality. On the other hand, the osmolarity is the number of osmotically active molecules in osmols per liter of solution. It gives a poorer indication of the osmotic conditions of a solution, since in this definition, the volume of the molecule of the dissolved substance in the solution is also integrated into the formula.

This volume is very variable. The difference between osmolality and osmolarity is quite considerable for concentrated solutions of a highly molecular substance, as for example, plasma with its protein content.

For biological purposes, the osmotic pressure of solutions of different substances is measured by the fall in the freezing point. It is given in milliosmol/kg water (mOsm/kg H_2O).

When the osmolality in the extracellular space rises, intracellular fluids flow into the extracellular space. Dehydration, fever, and death may result. The limits of plasma osmolality compatible with life lie between 250 and 350 mOsm/kg H_2O. A fall in the extracellular osmolality leads to the flow of water into the intracellular space with water intoxication. Brain edema, epileptic fits and death can then occur.

When the serum osmolality rises, water excretion through the kidneys is reduced to an absolute minimum. The thirst center is stimulated and the thirst sensation secures then an adequate water intake.

β) Water Reabsorption in the Proximal and Distal Tubules of the Nephron

The plasma undergoes simple filtration in the renal glomerula. Protein and formed elements of the blood are retained in the plasma, while water and all the normally present solutes pass on into the tubule with the so-called primary urine. In the human, the primary urine formed is 100 liters/24 h/m^2 body surface, or 180 liters daily for an adult of average body weight. The amount of solutes is 31 osmol/m^2 of body surface.

About 30 osmol solutes are actively reabsorbed from the isotonic primary urine in the proximal tubule. The amount of water in the isotonic solution passively reaches the peritubular bloodstream. This occurs by means of the "osmotic suction" which exists after the active reabsorption of the solutes. The proximal tubule expends no energy for osmotic work during this process of reabsorption since the end effect of the reabsorption is an isotonic solution. Overloading with osmotically active substances such as glucose, mannitol, and urea, etc. can decrease the reabsorption so that they have a diuretic effect. Reabsorption from the proximal tubule is otherwise dependent of the tubular volume (BRUNNER, 1966). So 15 liters of water and 1 osmol or 3% of the total solutes originally contained in the primary urine reach the ascending limb of Henle's loop.

The mammalian kidneys can limit the water loss from the body to the necessary extent through vasopressin-regulated water reabsorption in the distal tubules and the collecting ducts. The mammalian kidneys employ the countercurrent multiplier system for this variable water reabsorption. This system, which is used technically and has a wide spread throughout many animal species (SCHOLANDER, 1957), permits a high osmotic effect to be achieved with relatively low energy expenditure and the urine to be concentrated in the collecting ducts (WIRZ, 1960). In animals the ability to concentrate urine is dependent on the formation of Henle's loop. Beavers have a short Henle's loop and can concentrate the urine to 600 mOsm/kg. Rabbits, with long and short loops, can concentrate up to 1400 mOsm/kg, as can the human. The sand rats in the desert, however, with an exceptionally long loop and a singular renal papilla penetrating far into the ureter can survive without water and concentrate the urine to 20 times the serum osmolality, i.e. up to 6000 mOsm/kg (O'DELL, 1960). Sodium is actively reabsorbed in the ascending limb of Henle's loop, which is impermeable to water. The sodium is then released into the extracellular fluid of the surrounding tissues, as well as into the lumen of the descending limb of the loop. A high concentration of sodium thus circulates in the portion of Henle's loop lying towards the papilla and exerts a high osmotic pressure on the surrounding tissues. This pressure increases from the renal cortex towards the medulla, and in the human it is approximately 4 times higher in the papilla. The isotonic urine from the proximal tubule thus becomes first hypertonic at the tip of Henle's loop. Since, however, urinary sodium but no water is removed from the ascending limb of the loop, hypotonic urine reaches the distal tubules. In the absence of vasopressin, the epithelium of the distal tubules and of the collecting ducts is only slightly permeable to water, and the kidneys then excrete hypotonic urine. In antidiuresis, vasopressin is released. This opens the pores of the distal tubules and the collecting ducts to water so that the urinary water is passively withdrawn through the hypertonic medullary tissue in proportion to the osmotic gradient. "Free water" which is not bound osmotically is no longer excreted with the urine. The free-water clearance becomes zero or negative. The site of action of vasopressin lies in the epithelia of the collecting ducts and distal tubules. It is not known whether vasopressin also affects the descending limb of

Henle's loop by opening the pores to sodium or whether vasopressin promotes sodium reabsorption in the ascending limb (WIRZ, 1961). Adhesion of labeled pitressin can be demonstrated by autoradiography in the distal tubule and in the collecting ducts. However, autoradiographic methods are not possible in the thin segments of Henle's loop (DARMADY, 1960). In any case, under the influence of vasopressin, more sodium is retained in the renal medulla (LEVETIN, 1962). No vasopressin is in circulation during the state of maximal hydration and maximal diuresis. The distal tubules and collecting ducts are water impermeable. Hypotonic urine is transported uninfluenced through the hypertonic medullary tissue. In the state of maximal antidiuresis, the epithelia of the distal tubules and collecting ducts are freely permeable to water. Urinary water is removed in proportion to the osmotic pressure of the extracellular fluid of the medullary tissue until the two osmotic pressures are similar (GOTTSCHALK, 1959). This osmotic pressure in man is approximately 4 times the plasma osmolarity or 1 200–1 400 mOsm/l. Some authors (JAENIKE, 1961; 1964; GARDNER, 1964) suppose that vasopressin also increases the permeability of the collecting ducts to urea, which takes part in the circulation of the countercurrent system during antidiuresis and contributes to the hypertonicity of the medullary tissue (SCHMIDT-NIELSEN, 1958). This hypothesis, however, has been rejected by others (GRANTHAM, 1966).

An average antidiuresis is necessary for the excretion of an isotonic urine. Small quantities of vasopressin open the pores of the collecting ducts and of the distal tubules to such an extent that osmotic equalization with the hypertonic medulla is not fully achieved.

A second countercurrent-exchange system exists in the vasa recta of the renal medulla. In contrast to the epithelium of Henle's loop. the walls of the vasa recta are freely permeable to both water and electrolytes, so that the osmolality of the blood in the vasa recta is always the same as that of the surrounding tissue. The vasa recta supply the medullary tissue with oxygen and nutritives and remove the reabsorbed free water. The loop-shaped arrangement of the vessels prevents the loss of the high concentration of electrolytes in the medulla through the blood flow. Blood electrolytes from the ascending limb are transferred into the hypotonic blood in the descending limb, and water from the descending limb enters the ascending limb. During maximal diuresis the blood supply to the medulla is accelerated (THURAU, 1960). It is still undecided whether the increased medullary blood circulation leads to the removal of electrolytes from the medulla with a decrease in the medullary hypertonicity (the "wash-out effect", BUCHBORN, 1964), or whether the diminished medullary hypertonicity causes an increase in circulation. Highly concentrated urine cannot be obtained when the medulla is insufficiently hypertonic, despite the presence of vasopressin and the water-permeable epithelium of the collecting ducts. A maximal but unphysiologic antidiuresis can be achieved in addition to the effect of vasopressin by reduction of the glomerular filtration by less than 30%.

Factors Influencing the Antidiuretic Effect of Vasopressin. The antidiuretic effect of vasopressin usually decreases slowly after several days of excessive water intake, and the effect steadily increases again with limited water intake. This decrease in the concentration power is related on one hand to the loss of the medullary hypertonicity (the "wash-out effect") (THURAU, 1960; KRAMER, 1960; BUCHBORN, 1964). On the other hand, the concentration power may be affected by the thickening of the basal membrane of the pars recta of the proximal tubule and the following portion of Henle's loop due to the chronic hydration (PICK, 1966).

In newborns, vasopressin has no antidiuretic effect during the first 3 days of life, although endogenously formed vasopressin not attached to neurophysin circulates freely (DICKER, 1966). In nephrogenic diabetes insipidus vasopressin exerts no or only very limited antidiuretic effects (see p. 53). Hypercalcemia also causes a diminished antidiuretic effect, but here this is most probably explained by the obstruction of individual tubules (BANK, 1965). In addition, there are morphological changes in the epithelia of the distal nephron in hypercalcemia (CARONE, 1960), and the permeability of the cells to water may be decreased. The passive absorption of water from the collecting ducts thus remains inadequate (CARONE, 1960; MANITIUS, 1960).

Hypokalemia leads to tubular nephropathy with polyuria and hyposthenuria (RELMAN, 1956). In hypokalemia, the countercurrent mechanism is probably impaired by the diminished sodium transport in the ascending limb (BANK, 1964; BRUNNER, 1966). Using the model of a toad's bladder, it was shown that a normal ionic concentration is necessary for the full effect of vasopressin (SCHWARTZ, 1967).

Maximal diuresis is not possible in adrenal insufficiency and the capacity for concentration may sometimes be limited. Diminished sodium reabsorption with insufficient medullary hypertonicity is probably responsible for the impaired concentration process. The disturbance

in diuresis is attributable to the decreased glomerular filtration, the fall in the extracellular fluid volume, and in particular, to the loss of water impermeability in the collecting ducts (KLEEMAN, 1964) (see p. 305); the increase in vasopressin secretion due to the lack of glucocorticoids may also be a contributory factor (DINGMAN, 1960).

High doses of parathormone and vitamin D result in resistance to vasopressin. This effect is not only due to the hypercalcemia, but in particular to the decrease in sodium reabsorption and an insufficient sodium-urea concentration in the renal medulla (EPSTEIN, 1959; PICKFORD, 1966).

When the amount of solute is constant, the volume of urine is inversely proportional to the release of vasopressin. If this hormone is absent, the urine volume is proportional to the quantity of the solutes. The solutes are related to the diet as well as to the amount of the body's breakdown products. The quantity of the endogenous solutes can be reduced to 200 mOsm per day with suitable carbohydrate intake. Only 135 cm^3 of urine with an osmolality of 1500 mOsm/kg H_2O is necessary to get rid of this amount. Consequently, this is the smallest possible physiological urine volume.

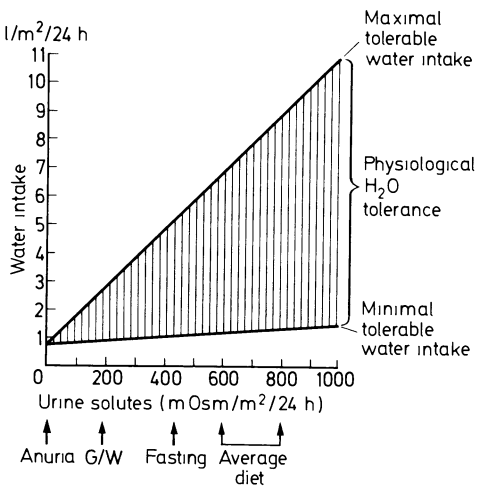


Fig. 3. Limits of the tolerable water intake related to the diet (according to KERRIGAN, 1955) (G/W: glucose-water intake)

Minimum and maximum tolerable water intake is therefore dependent on the quantity of the solutes and can be estimated from the fixed osmotic limits of the urinary volume after correction for the water loss due to insensible perspiration and water formed by oxidation (KERRIGAN, 1955).

Vasopressin exerts its antidiuretic effect on the denervated and isolated kidney. Contrary to suggestions (GINETZINSKY, 1958), the mode of action in the distal tubules and collecting ducts is not related to the liberation of hyaluronidase (BERLYNE, 1960). It is due either to a chain reaction between the disulfide links of vasopressin and the epithelial SH-radicals opening the pores (SCHWARTZ, 1964) although oxytocin derivatives without disulfide links also exert an antidiuretic effect, or to the enhanced formation of cyclic 3′,5′-AMP (ORLOFF, 1967; GRANTHAM, 1966). The action of vasopressin is independent of oxidative metabolic processes and is not related to a protein or enzyme system since puromycin and actinomycin D do not inhibit its action (RASSMUSSEN, 1960). Using the model of the toad's bladder in which the epithelium behaves like that of the collecting ducts, it has been demonstrated that vasopressin makes a dense impervious layer permeable to water and urea, whereas a second layer impermeable to dissolved substances can only be affected by amphotericin (LEAF, 1967). Vasopressin in physiological doses has no direct effect on electrolytic excretion, but in unphysiologically high doses, it has some influence through alterations in blood pressure and changed filtration conditions.

Physiological amounts of vasopressin do not directly influence glomerular filtration, the renal plasma flow or the different clearances, with the exception of the free water clearance. An extrarenal effect of vasopressin resulting in a shift of electrolytes from the extra- into the intracellular space has not been proven.

γ) Other Effects of Vasopressin

Vasopressin causes capillary and arteriolar constriction in the skin, the connective tissue, the internal organs, the smooth muscle of the gastrointestinal tract, the myocardium, and to a lesser degree in the musculature. The arterioles of the splanchnic area are particularly affected. Capillary constriction alone, however, does not explain the increase in the blood pressure. The effect on the blood pressure due to extracts of the posterior pituitary is probably of no physiological significance. It would seem more likely that vasopressin does produce a pharmacological action, since even in diabetes insipidus the blood pressure is normal. Vasopressin exerts a CRF-like effect on the anterior pituitary (see p. 300) which is probably of no physiological importance, but which can be employed diagnostically (see p. 388). Nor is the ACTH-like effect on the dog's adrenals (HILTON, 1960) of any physiological significance in man. This also applies to the other actions of vasopressin; namely, the insulin-like effect, the antilipolytic action on

adipose tissue (MIRSKY, 1963; BALASSE, 1966) and the glycogenolysis in the liver with hyperglycemia achieved by high doses of vasopressin in the dog (BERGEN, 1960). It has been suggested that in addition to its antidiuretic effect, vasopressin also influences the kidneys through the thirst center (PASQUALINI, 1959). From clinical observations, however, this appears improbable. There have recently been suggestions that vasopressin may have an effect on the transport of water and sodium in the small bowel. Either more water and sodium are secreted into the lumen of the bowel under the influence of vasopressin or the active transport of sodium from the lumen is inhibited (SOERGEL, 1968).

4. Interrelations

a) Anterior Pituitary Gland

Normal nutritional and electrolytic intake and metabolism are necessary for polyuria to become manifest. With failure of anterior pituitary function this is no longer possible. Polyuria disappears and becomes apparent again only after adequate replacement therapy.

Glucocorticoids, thyroid hormones and growth hormone promote renal blood supply and glomerular filtration. Lack of glucocorticoids causes a change in the cell permeability with a shift of the extracellular fluid into the intracellular space. The permeability of the collective ducts cannot then be reduced. Thus, polyuria disappears in diabetes insipidus subsequent to failure of the anterior pituitary gland. It is unnecessary to invoke a previously postulated anterior pituitary substance, "diuretin", which does not exist.

b) Deoxycorticosterone – "Pseudodiabetes Insipidus"

A syndrome similar to diabetes insipidus can be produced in the dog with high doses of deoxycorticosterone. Only polyuria, polydipsia, and the low specific gravity of the urine are common to "pseudodiabetes insipidus" and genuine diabetes insipidus. In contrast to the state of dehydration in diabetes insipidus, there is over-hydration in the "pseudo" syndrome, and the polyuria is uninfluenced by vasopressin but is affected by salt withdrawal. In true diabetes insipidus, polyuria can be decreased only to a limited extent by salt withdrawal. Deoxycorticosterone causes reabsorption of abnormally large amounts of sodium but also its increased filtration – the "escape phenomenon". Because of this, the extracellular fluid increases. Vasopressin secretion is insufficient and there is a simultaneous sensation of thirst which leads to polydipsia and to secondary polyuria. The low specific gravity of the urine is due to the total sodium reabsorption. In contrast with vasopressin, deoxycorticosterone has no influence on water metabolism when salt has been removed from the diet.

E. Hypofunction of the Hypothalamic-Neurohypophyseal System: Diabetes Insipidus

1. Definition

Diabetes insipidus is a disease characterized by the inability to concentrate urine and by polyuria of 5 to 20 liters daily with corresponding polydipsia. The disease is due to disorders in the formation or release of vasopressin. Nephrogenic diabetes insipidus is not due to the lack of vasopressin, but to a failure of the distal tubules to respond to vasopressin.

2. Incidence

Diabetes insipidus is an uncommon disease. It occurs somewhat more frequently in males than in females. It arises during the first decade of life in almost half the patients. In the Zurich Medical in-patient clinic (1958–1968), the disease was found 28 times in 35164 patients, with a clear preponderance of the symptomatic forms.

3. Etiology, Pathology, and Pathogenesis of Diabetes Insipidus

a) Etiology

Table 4 gives a summary of the different causes of diabetes insipidus.

The relative incidence of the different etiologies is given in Table 5.

b) Pathogenesis of the Symptomatic and Idiopathic Diabetes Insipidus

"Central" diabetes insipidus is caused by inadequate water reabsorption in the distal nephron due to the absence of vasopressin. It is still unknown to what extent the disorder is due to impaired formation or release of vasopressin. It is also unknown whether two different types of diabetes insipidus can be differentiated on this basis. It is probable that in "idiopathic" diabetes insipidus, there is a failure of the osmoreceptors. Although it has been suggested that diabetes insipidus may result from increased

Table 4. The different causes of diabetes insipidus

I. Failure of vasopressin	II. Vasopressin ineffective
1. Damage to the hypothalamo-neurohypophyseal system, symptomatic diabetes insipidus	1. Nephrogenic hereditary, vasopressin-resistant diabetes insipidus
a) Tumors, in particular cysts, craniopharyngioma, gliomas, tumors of the anterior pituitary, metastases, storage granulomas (HAND-SCHÜLLER-CHRISTIAN)[a]	
b) Inflammatory diseases: encephalitis, meningitis, granulomatous processes such as TB, syphilis, sarcoidosis, lymphocytic neurohypophysitis (SAITO, 1970)	
c) Degenerative, in particular vascular destruction	
d) Traumatic damage	
e) After therapeutic hypophysectomy	
2. Hereditary diabetes insipidus, morbid anatomy mainly negative	
3. Primary "idiopathic" diabetes insipidus, unknown etiology	

[a] The Sternberg-Priesel's nodules which are typical to the posterior pituitary and pituitary stalk can occasionally form larger tumors described as tumorettes. They can, but need not give rise to endocrine disorders.

inactivation of vasopressin in the liver and kidneys (HANKISS, 1961), there has so far been no experimental support for this. The human can form antibodies to posterior pituitary extracts (ROTH, 1966); however, their clinical significance has not been evaluated. Disturbances in the regulation of diuresis become obvious when more than 85% of the neurons in the hypothalamic-neurohypophyseal system are lost. The full clinical picture does not appear until there is 95% failure.

An intact anterior pituitary and its dependent endocrine glands must be present before polyuria and the complete picture of diabetes insipidus can develop (see p. 48) since failure of these glands leads to disturbed renal function. Glomerular filtration may then decrease, and cortisol is necessary to "seal off" the collecting ducts so that maximal diuresis can occur. In addition, the responsive threshold of the osmoreceptors is raised by cortisol (AUBRY, 1965). Reduced nutritional intake may also have some effect in this regard. It has been observed that polyuria regresses when the posterior pituitary process attacks the anterior pituitary. The renal inability to concentrate continues despite the reduced polyuria, and consequently, diabetes insipidus may first become apparent when cortisone is substituted in failure of both pituitary lobes.

A concentrating power of up to 1016 may be maintained after selective loss of the pituitary without destruction to the hypothalamus such as occurs after surgical removal of the pituitary gland. From this it can be deduced that vasopressin can be further secreted under these conditions. Secretory transmitting fibers of the supra-optic and paraventricular nuclei partly terminate at the vessels of the pituitary stalk and immediately under the ependyma. Fibers of the supraoptic-neurohypophyseal tract may regenerate after division and form a sort of replaced posterior lobe with blood vessels of the proximal stump (BARGMANN, 1968). See p. 52f. for the three phases of the course of postoperative diabetes insipidus. For the rare coincidence of diabetes insipidus and SHEEHAN'S syndrome (AGUILÒ, 1969), see Chap. V, p. 93.

Table 5. Etiological classification of 124 cases of diabetes insipidus. [According to BLOTTNER: Metabolism **7**, 191 (1958)]

Idiopathic	45
Primary brain tumor	36
Metastatic brain tumor	3
Syphilis	7
Hereditary diabetes insipidus	3
Postencephalitic	3
Xanthomatosis	2
Myeloid leukemia	2
Infectious chorea	2
Cranial fractures	3
Malignant lymphoma	1
Sarcoidosis	1
Cerebral arteriosclerosis	1
Birth injury	1
Calcification of the internal carotid artery	1
Postvaccinal	1
Basal arachnoiditis	1
	124

4. Clinical Features and Symptoms of Idiopathic and Symptomatic Diabetes Insipidus

a) History

Polyuria and subsequent polydipsia can arise overnight or may develop gradually. Polydipsia and enuresis can occasionally precede the

ability to concentrate urine and if these persist for a long time, sometimes result in children being unfairly labeled neurotics (FANCONI, 1956). According to SMITH (1951), 50% of patients with diabetes insipidus excrete a urine volume of between 4 and 8 liters; 25% of patients excrete between 8 and 12 liters. A urinary output of up to 40 liters has been described in the literature. This amount of urine is theoretically possible. Within individually set limits, however, the volume of urine is largely dependent on the serum content of the substances to be excreted in urine. Thirst caused by fluid withdrawal has a compulsive character. Patients drink everything available to them, even their own urine. If no fluid is replaced, polyuria continues leading to dehydration with dry tongue, fever, delirium, and collapse. In a baby, an unexplained fever is often the first symptom of diabetes insipidus. This thirst fever is characterized by the fact that the maximum rise is usually found in the mornings, in contrast to fever due to inflammations.

Possible causes of a lesion in the hypothalamic region must be precisely searched for while taking the case history. Inflammatory diseases and tumors must be excluded. The family history must be investigated. In contrast to the severe mental disorders due to failure of the anterior pituitary, insufficiency of the hypothalamo-neurohypophyseal system does not lead to severe psychological disturbances. Thirst is an immediate result of the excessive loss of water, and it is pointless to interpret the thirst of a patient with diabetes insipidus as a deep-seated psychological disturbance of the instinctual sphere. BLEULER (1954) clearly and decidedly objects to the psychiatric approach of diagnosis and therapy of diabetes insipidus.

b) Symptomatology

Apart from the dry skin and frequently dehydrated mucous membranes, examination of the patient does not reveal any special features.

Polyuria with great water loss which cannot be rapidly compensated through adequate intake results in a series of indirect symptoms: constipation, inadequate saliva flow, absence of sweat secretion. Sleep disturbances due to the thirst can cause the patient to be irritable and to show neurasthenic symptoms. Polyuria and polydipsia do not have any negative effects on kidneys and circulatory organs even after decades.

In the presence of a destructive process in the hypothalamus, diabetes insipidus can be associated with disturbances in the visual fields, signs of total or partial insufficiency of the adenohypophysis, pressure symptoms on the brain, and other hypothalamic syndromes (see p. 32). Even in the absence of neurological symptoms, all patients with diabetes insipidus should at least have an X-ray of the skull and a lumbar puncture and possibly a pneumoencephalography to exclude the symptomatic form.

Features due to the lack of oxytocin are not as a rule found in association with diabetes insipidus. Even pregnant women suffering from diabetes insipidus give birth normally.

c) Findings in Urine and Plasma

The most important laboratory finding is a 24-hour urinary output of over 4 liters of clear urine, devoid of sugar and protein, and with a specific gravity which seldom exceeds 1005. The urinary sodium chloride concentration is low, although the amount of NaCl in the 24-hour output is normal. The urine is almost always hypotonic and its osmolality does not exceed that of the plasma, 300 mOsm/kg H_2O (see p. 46). In the absence of vasopressin, the free water clearance which reflects the 'renal excretory power for free, as against osmotically-bound water is usually positive. Estimation of the plasma osmolality can differentiate between diabetes insipidus and polydipsia. The test is carried out in the morning in the fasting state and with free water intake, or better still, after 6–8 hours of water deprivation. The plasma osmolality in diabetes insipidus is 300 mOsm/kg H_2O or more, whereas in polydipsia, it is under 295 mOsm/kg H_2O (ROMANI, 1965). The plasma osmolality is always higher than the urine osmolality in diabetes insipidus after 8 hours of water deprivation (PRICE, 1966).

5. Special Forms of Diabetes Insipidus

a) Diabetes Insipidus after Injury, Therapeutic Hypophysectomy or after Section of the Pituitary Stalk

Experiments in animals have shown that loss of more than 85% of the neurons in the hypothalamic-neurohypophyseal system gives rise to a partial diabetes insipidus, whereas failure of more than 95% is necessary for the development of a full-blown diabetes insipidus. Similar observations were made after therapeutic hypophysectomy for treatment of carcinoma of the breast and prostate and in diabetic retinopathy. The resultant diabetes insipidus is usually mild, presents special symptoms, and runs a special course. The urinary output varies from day to day and is usually between 3–4 liters. The

average specific gravity is 1010, but the concentration power may reach 1016. The Hickey-Hare test using hypertonic saline infusion is usually pathologic but can sometimes be normal. In the majority of cases, diabetes insipidus does not develop after transsphenoidal subdiaphragmatic hypophysectomy. The risk of diabetes insipidus is not predictable in transfrontal hypophysectomic or division of the pituitary stalk. Furthermore, there is no relation between the completeness of the hypophysectomy and the severity of the diabetes insipidus that may result.

Three phases have been observed in animal experiments and after therapeutic hypophysectomy in man.

1. Immediate polyuria, arising within minutes or hours of the lesion and continuing for hours or days.

2. The intermediate phase with normal urinary output, which can last for a few hours to 3 days.

3. The permanent state with diabetes insipidus of variable severity. All combinations of the three phases are possible. The immediate polyuria can disappear, and permanent polyuria may arise days later without going through phase 1 or phases 1 and 2.

It is assumed that phase 1 is due to a release disturbance caused by the traumatic shock to the neurohypophysis. The polyuria subsequently regresses due to the mobilization of vasopressin (KOVACS, 1962). This continues until phase 3, when the increasing degeneration of the supra-optico-neurohypophyseal tract reaches the nuclei, resulting in destruction (SHARKEY, 1961).

Experience has taught us that treatment is not always indicated in the transitory first phase. The treatment of the permanent phase depends on the degree of polyuria.

Vasopressin may be produced; however, loss of the neurohypophysis, the storage organ of vasopressin, causes a disturbance in the release mechanism.

b) Hereditary Central Diabetes Insidipus

The hereditary form of diabetes insipidus it not common. At present, there are only 25 families known to be suffering from this inherited disease.

Usually no patho-anatomical changes are found in the hypothalamus. Hypoplasia of the ganglion cells of the supra-optical and paraventricular nuclei is claimed to have been found in only a few cases.

The disease is inherited by a simple dominant gene. There is a 1:1 ratio between the diseased and healthy descendants. The male sex is more prone to show manifestations of the disease, and 64% of the cases have occurred in males. The reason for the less frequent manifestation in women is not known (MARTIN, 1959). The disorder usually becomes apparent in very early adolescence.

In addition to the simple dominant inheritance, families are known which have sex-linked recessive inheritance, where only the male shows manifestations of the disease (FORSSMAN, 1955). A third mode of inheritance through an autosomal recessive gene has been described in rats (VALTIN, 1967).

In contrast to the nephrogenic diabetes insipidus, there is no vasopressin resistance.

Consequently, the disorder is due either to a disturbance in the osmoreceptors (MARTIN, 1959) or, according to limited postmortem findings, to atrophy of the supra-optical nucleus and the neurohypophysis. In the inherited diabetes insipidus described in rats, hypertrophy of the system was found (VALTIN, 1967).

c) Nephrogenic, Vasopressin-Resistant, Hereditary Diabetes Insipidus

This uncommon congenital and hereditary form of diabetes insipidus usually exhibits pronounced polyuria, polydipsia and hyposthenuria. The disease becomes apparent soon after birth, although the typical symptoms can be masked in the infant by dehydration, fever, vomiting, and convulsions (GAUTIER, 1956). Experienced mothers make these infants drink, holding them directly under the tap ("water-babies").

In the fully-developed illness, the urinary output in the adult is always more than 8 liters. It is, however, dependent upon osmotic load and especially the sodium intake. The urine osmolality lies between 50 and 100 mOsm/kg H_2O. Under osmotic stress, it should never rise above 280. The specific gravity is usually 1005 and 1010 in osmotic diuresis. All the men observed presented the full picture. In the few affected women, the full-blown disorder was present or there was only a mild form with a concentration power of up to 1019. In addition, acquired hyperuricemia and occasionally gout have been observed (GORDEN, 1971).

α) Pathogenesis

Vasopressin is produced and released normally; moreover, it is not metabolized rapidly and can be demonstrated in both plasma and urine of these patients (HOLLIDAY, 1962/63). However, there is a lack of response in the distal nephrons to endogenous and exogenous vasopressin, as well as to the synthetic lysin-vasopressin. Uri-

nary flow and urine osmolality are not influenced in any way by concentrations of vasopressin which are high enough to exert definite side effects on the vasomotor system and the gastrointestinal tract. No morphological changes are found at post mortem. The significance of a shortened proximal tubular segment, which has been observed on two occasions, is unknown (McDonald, 1955). Occasionally hydronephrosis and bladder hypertrophy have been observed, resulting from the voluntary urine retention with the polyuria. The acquired hyperuricemia occurring only in adults is due to a low endogenous uric acid clearance, probably connected to the low renal plasma flow and high filtration fraction (Gorden, 1971).

β) Differential Diagnosis

All conditions of nephrogenic polyuria come into the differential diagnosis (see Table 6, p. 56). The response to vasopressin in all the other conditions of nephrogenic hyposthenuria is merely diminished and not abolished.

γ) Genetics

Heterozygous women transmit the inheritance on the X chromosome. The expressivity of the disorder is variable. Men become clinically ill. The women are carriers but can also demonstrate abortive forms of the illness. In an investigation of a family of Ulster Scots which came to Nova Scotia in 1761 on the ship Hopewell, over 9 generations confirm this mode of inheritance. The folklore relates the illness to a curse of a gipsy (Bode, 1969). It is assumed that in certain families inheritance is autosomal. However, this has not been definitely proven.

δ) Therapy

It is most important to allow the patient to drink as much as he needs. The danger is acute for infants since brain damage can result from repeated dehydration. When the patient is inconvenienced by the frequent drinking and urinary output, it is advisable to restrict the salt in the food and to give thiazide-diuretics as well with concurrent potassium and allopurinol (Gordon, 1971) administration. The procedure is described on p. 60ff.

In acute dehydration when the patient is unable to drink, a hypotonic glucose solution should be given intravenously. A 2.5–3% glucose solution should be used, since this causes neither hemolysis nor a temporary rise in the plasma osmolality due to osmotic diuresis as would occur with a 5% glucose solution.

d) Lithium-Induced Nephrogenic Diabetes Insipidus

Lithium carbonate is widely used in the treatment and prophylaxis of manic-depressive disease. In man and in animals, chronic administration application of lithium salts can cause an insensitivity of the kidneys to vasopressin and lead to a reversible nephrogenic diabetes insipidus. Similar observations have been made with magnesium, manganese, and caesium. Whether these salts act by inhibiting activation of cyclic AMP or by a similar mechanism is not yet decided (Editorial 1972; Singer, 1972).

e) Occult Diabetes Insipidus (see also p. 55)

The inadequate fluid intake and resultant small urinary output differentiate the occult diabetes insipidus in babies and small children from the usual diabetes insipidus. In babies the thirst center is at first hyposensitive, and there is a particular tendency to become dehydrated and to develop fever and a hyperelectrolytemia. These cases often turn out to be nephrogenic diabetes insipidus. As the patient grows older, the thirst mechanism gradually or suddenly begins to function. This usually occurs after the 10th month. The occult diabetes insipidus is then converted into a manifest diabetes insipidus. Disturbances of the thirst center must be distinguished from true diabetes insipidus with inadequate fluid intake (see p. 55).

f) Transitory Diabetes Insipidus

A few cases of transitory diabetes insipidus have been observed (Kedes, 1964). The pathogenesis is unexplained. Although antibodies to lysin-vasopressin can occur in man, none have been demonstrated in this form of diabetes insipidus (Roth, 1964). It has been suggested that diabetes insipidus can arise from increased breakdown of vasopressin (Hankiss, 1961). This, however, will remain uncertain until such cases are reinvestigated with sensitive radioimmunoassays for vasopressin.

6. Primary Polydipsia

A primary irritation of the hypothalamic thirst center leads to excessive water intake of up to 20 liters with consequent polyuria. This is most often due to neurotic factors (dipsomania, potomania) and seldom to an organic lesion. Neurotic polyuria is usually connected with changes in character and psychic disorders, such as hysteria. Four-fifths of the patients are

women. The polydipsia usually develops gradually over weeks or months and may run an intermittent course. Character changes due to the illness may, however, also occur in true diabetes insipidus (ANGST, 1963). Therefore, it may be very difficult to differentiate primary polydipsia from diabetes insipidus, and this is usually only possible after several days of hospitalization or by observation of the course. It is particularly difficult to establish the diagnosis since persistent polyuria of more than three days duration, as well as over-hydration may lead to a relative renal vasopressin resistance. Estimation of the plasma osmolality in the morning in the fasting state but with free water intake reveals an important difference. Patients with diabetes insipidus are usually somewhat dehydrated after a night of unbroken sleep, and the plasma osmolality is more than 300 mOsm/kg H_2O. In polydipsic patients the plasma osmolality is slightly reduced. Therefore, the fasting plasma osmolality of patients with diabetes insipidus is generally different from that of polydipsic patients. The osmolality of both types of patients can, however, overlap with normal values. Estimation of the plasma osmolality after 6–8 hours of water restriction would permit an even more definite differentiation between diabetes insipidus and polydipsia (ROMANI, 1965). The water deprivation test alone, or the Hickey-Hare test alone, can give misleading results since in the latter test, a sufficient hypertonicity of the diluted plasma cannot be attained. In the water deprivation test lasting 24 hours or longer the overhydrated patient excretes excess water with a loss in weight. Antidiuresis cannot commence in either of these two tests. Even when the water deprivation test is continued long enough for a normal or elevated plasma osmolality to be reached, the renal response to vasopressin, in the presence of intact osmoreceptors, is only insufficient in polyuric conditions. A comparison between the concentration capacity in the water deprivation test and after intravenous vasopressin can help here. When carried out under the same osmotic conditions, a urine concentration which is higher in the water deprivation test than after intravenous vasopressin indicates polydipsia. A concentration which is higher after intravenous vasopressin than in the water deprivation test suggests diabetes insipidus (BARLOW, 1959). The depot-pitressin test usually gives a more reliable result. It is carried out as follows: fluid intake is unrestricted. On the morning of the first day of the test, a placebo, such as sesame oil, is given intramuscularly. The urinary output of patients with diabetes insipidus is uninfluenced. The fluid intake decreases in some dipsomanic patients. On the following morning, when the fluid intake and urinary volume remain unaltered, 5 units of pitressin tannate in oil are injected intramuscularly. In diabetes insipidus, the polyuria is reduced, and with suitable intake, the patient is clinically improved. The dipsomanic patient will, however, continue to drink, will feel unwell, will put on weight until the signs of water intoxication develop: nausea, headache and epileptic fits. In some polydipsic patients, however, the feeling of thirst may disappear once a certain overhydrated state has been reached. The "dipsostat", the thirst center, is set for overhydration, i.e., for a lower osmolality.

Organic polydipsia can usually only be suspected during life when it occurs together with other hypothalamic symptoms such as fever, emotional changes, and partial anterior pituitary deficiency. Polydipsia or adipsia may occur in hydrocephalus (HAGEN, 1967). Organic polydipsia can be combined with a true, or possibly a partial, diabetes insipidus. The diagnosis can then be very difficult.

7. Adipsia, Usually Associated with (Partial) Diabetes Insipidus

(Central Hyperelectrolytemia Diabetes Insipidus Occultus Hypersalemicus FANCONI*)*

In the few cases in which an organic lesion has been definitely diagnosed, polydipsia as a symptom of the irritation has preceded the later *failure* of the thirst center by years. Tumors have been described as the cause of this hypothalamic lesion, particularly often ectopic pineoloma. Traumatic hemorrhage, and inflammation are other causes. The disturbed realization of the feeling of thirst, adipsia, results in so-called central hyperelectrolytemia. The serum sodium may lie between 160–180 mEq/l, corresponding to an osmolality of 320–360 mOsm/kg. The increase of plasma electrolytes is not unlimited, but a new state of equilibrium with relative and absolute hypertonic dehydration becomes established. The plasma volume is unaltered. Intra- and extracellular fluid volumes are decreased. Normal conditions can be re-established by forced fluid intake with simultaneous vasopressin administration. The previous false adjustment is immediately resumed on the withdrawal of the vasopressin and water. Regulation of the volume is still intact, whereas osmoregulation is disturbed. An additional rise in the osmolality does not result in increased release of vasopressin so that in the majority of cases, a partial diabetes insipidus is also present. Adipsia and diabetes insipidus together

produce the occult hypersalemic diabetes insipidus (FANCONI, 1956). In the baby and small child the perception of thirst is only slight. Diabetes insipidus can therefore easily lead to dehydration and hyperelectrolytemia, that is to say, an occult diabetes insipidus can develop in the absence of an organic lesion in the thirst center. On the other hand, hemorrhage and thrombosis of the brain can produce hypernatremia, especially in infants (COOKE, 1960). The diagnosis of organic adipsia or central hyperelectrolytemia must only be made when clouding of consciousness and intellectual defects can be excluded as causes of the disturbed water intake.

8. Differential Diagnosis of Diabetes Insipidus

In a typical case, the diagnosis can be made simply from the history. However, it may be difficult to differentiate the disease from psychogenic or organic polydipsia, since they both lead to vasopressin hyposensitivity. In addition to diabetes insipidus in organic hypothalamic lesions, there may be symptoms due to stimulation and failure of the thirst center which complicate the picture. Finally, there may be partial diabetes insipidus in the presence of an insufficient vasopressin secretion.

Diabetes insipidus must be considered when the 24-hour urinary output exceeds 4 liters. The simple measurement of the urinary volume in patients who believe they excrete an enormous amount of urine can often exclude the diagnosis of diabetes insipidus. In general, the specific gravity of the urine in diabetes insipidus is under 1005 corresponding to an osmolality of < 200 mOsm/kg H_2O. The upper limit for the specific gravity is usually taken as 1008. The urine is hypotonic, its osmolality is lower than that of the plasma (300 mOsm/kg H_2O). The free water clearance is negative. It is, however, possible that slightly hypertonic urine with a correspondingly high specific gravity may be produced under particularly pathological conditions where the glomerular filtration is greatly reduced, even in the absence of vasopressin (BERLINER, 1967; KLEEMAN, 1957). Definite diagnostic border-line values for urinary outputs and concentration capacities cannot be given because of the occurrence of partial diabetes insipidus, particularly the type which develops postoperatively.

The polyuria in diabetes mellitus rarely exceeds 4 liters. The specific gravity is high and glucosuria is present. The daily urinary output in chronic nephritis and cystic kidneys is seldom more than 4 liters.

Further, nephrogenic polyuria occurs in hypokalemia with hyposthenuria (see p. 48) and also in primary aldosteronism (see p. 332). Finally, polyuria may result secondary to hyper-

Table 6. Differential diagnosis of polyuria. [Modified according to KLEEMAN *et al.*: Arch. int. Med. **101**, 1023 (1958)]

Mechanism:		
Water	Excessive water intake	Polydipsia a) Psychogenic b) Organic (seldom)
	Incapacity of tubular reabsorption	Vasopressin *deficiency* or disturbed release: Diabetes insipidus
		Disturbed *response* of the nephrons to vasopressin: Nephrogenic, hereditary diabetes insipidus Polydipsia > 3 days Hypokalemia Primary aldosteronism Nephrocalcinosis Hyperkalemia Lithium medication or intoxication Manganese, magnesium or cesium intoxication Condition after urinary tract obstructions Multiple myeloma Amyloidosis
Dissolved substances:		
Glucose		Diabetes mellitus Phlorrhizin diabetes
Mannitol		Mannitol diuresis
Salts, especially NaCl		Chronic pyelonephritis, interstitial nephritis Salidiuretics, Hg-diuretics
Urea		Chronic renal disorders

parathyroidism and hypercalcemia, in which case the urinary output seldom exceeds 4 liters.

The same amount of urine is excreted daily in nephrogenic and in true diabetes insipidus. Nephrogenic diabetes insipidus is recognized by its failure to respond to vasopressin.

Neurotic or organic polydipsia can present the greatest difficulties in differential diagnosis. Often the prognosis and treatment can only be decided upon after hospital observation or by following the course of the disease.

9. Diagnosis of Diabetes Insipidus

Tests for Investigating Polyuria and Polydipsia

The diagnostic value of all of the following tests is limited by the fact that after more than 3 days of persistent water diuresis, the concentrating ability of the kidney decreases. This means that a high specific gravity cannot be achieved regardless of whether the water deprivation test, Hickey-Hare test, the vasopressin, or the nicotine test is employed. Maximal concentrating ability can only be examined after several days of water restriction or after treatment with depot-pitressin.

The least inconvenient and most reliable tests are still the water deprivation test under osmolality control with termination after 3–5% loss in weight and the vasopressin test. The Hickey-Hare and the nicotine tests are usually dispensable.

a) Water Deprivation Test

α) Principle

The plasma osmolality rises during water deprivation. In the healthy subject, the hypothalamic osmoreceptors are stimulated and vasopressin is then released, causing the pores in the distal nephrons to open for water reabsorption. Urinary flow falls; the specific gravity and urine osmolality rise. In diabetes insipidus, because vasopressin is absent, water continues to be excreted with development of dehydration and weight loss.

β) Method

7:00 a.m. Breakfast with little fluid.

7:30 Bladder emptied. Specific gravity and, when possible, osmolality of the urine are measured. Weigh patient hourly.
Blood collection for Hb, hematocrit, Na, Cl, when possible, osmolality, perhaps K and proteins. From 7:30 stop fluid intake. Watch patient closely. Collect urine in as many portions as possible. Note amount and specific gravity. Estimate osmolality whenever possible. After 2–3 hours or when patient can no longer urinate every half hour, only the hourly output is measured.

10:30 Blood for same determinations as above. Weigh patient.
The water deprivation test should last at least 6 hours, or better still, 24 hours. When the patient has lost 3–5% of the body weight, the test must be discontinued promptly.
Before the end of the test, blood collection is repeated. Patient is weighed again.
A sedative may be used to prolong the test.

γ) Interpretation

Diabetes insipidus is probable when: the urinary volume does not decrease or decreases only slightly during the test, when the specific gravity is not more than 1008, when the urine osmolality does not rise beyond 300 mOsm/kg H_2O, and when the patient loses 3–5% of his body weight. However, a patient with polydipsia, who at the beginning of the test is very highly overhydrated, can excrete urine with a low specific gravity for a long period of time due to the water retained in the tissues.

b) Hickey-Hare Test

(Infusion of Hyperosmolar NaCl-Solution)

α) Principle

Infusion of 2.5% NaCl causes an increase in the serum osmolality, and thereby stimulation of the osmoreceptors with release of vasopressin.

β) Method

Prepare: approximately 4 liters of unsweetened tea, 1 liter sterile 2.5% NaCl solution with complete drip set.

7:00 a.m. Breakfast with 20 ml fluid per kg body weight. This corresponds to 1.4 liters fluid for a 70 kg patient. No coffee. Smoking forbidden. Fluid should be ingested between 7 and 7:30.

From 7:15 Patient should void urine every quarter hour. The amount excreted

is measured, and the same volume of tea is ingested. When patient cannot void urine regularly, catheter must be introduced. Note the amount and specific gravity of each portion of urine.

10:00 Blood collection for Na, Cl, protein, Hb, hematocrit, when possible, serum osmolality.

Immediately after blood collection, infusion is started at a rate of 0.2 ml/kg body weight per minute, i.e. a 70 kg patient receives 14 ml/min = 280 drops per minute. The infusion should be continued for 45 min so that a 70 kg patient would receive a total of 630 ml.

It is practically impossible to count the drops. The quantity of fluid which should run off in the fixed time is best measured on the infusion bottle, and the rate adjusted accordingly. It may be convenient to employ an infusion pump for this purpose. A relatively large needle must be used in order to ensure rapid flow.

10:45 Stop infusion. Blood estimation as above.

The test is discontinued as soon as the urinary output collected during the test reaches the same quarter-hour volume as before the infusion was started.

γ) Interpretation

The estimation of Na, Cl, Hb, hematocrit and proteins will indicate whether the test is being carried out correctly. During the infusion, Hb, hematocrit and protein concentration in the serum decrease, whereas Na and Cl rise. Serum osmolality may be measured directly. A patient with polydipsia can be so hypo-osmolar at the beginning of the test that the infusion causes no hyperosmolality. In this case the test must occasionally be repeated with more NaCl.

Diabetes insipidus is probable when there is no decrease in urinary output and no increase in the specific gravity during the test.

An osmotic diuresis can occasionally simulate a false pathologic result. Simultaneous measurement of the free water clearance prevents false interpretations. In patients with circulatory disturbances there is a risk of pulmonary edema due to overloading with NaCl (15 g for 70 kg body weight).

c) Vasopressin Test

α) Principle

An injection of synthetic lysin-vasopressin can show whether the kidneys react normally to this hormone. Nephrogenic diabetes insipidus can then be diagnosed or excluded.

β) Method

The vasopressin test is carried out after the Hickey-Hare test so that the patient need not be hydrated again.

If the test is done on another day, the patient must be hydrated as in the Hickey-Hare test with 20 ml fluid per kg body weight.

Thereafter, the patient must void urine every quarter hour, and the same volume of tea is given to the patient. Note the quantity, specific gravity, and when possible, the osmolality of every urine sample.

At the beginning of a quarter-hour period, 1 IU lysin-vasopressin is given rapidly intravenously.

The blood pressure should be measured before and after the injection of vasopressin. The vasopressin test is not admissible in patients with hypertension and coronary disease, since vasopressin may cause a rise of approximately 50 mm Hg in the blood pressure.

γ) Interpretation

If the patient under investigation does not react with a decrease in urinary output and a rise in the specific gravity, the same amount of vasopressin should be tested in a normal subject.

If the normal person reacts to the vasopressin, then the patient who did not react must have a nephrogenic diabetes insipidus.

Central diabetes insipidus is present when the specific gravity and the urine osmolality are higher in the vasopressin test than in the water deprivation test. A high specific gravity in the water deprivation test indicates polydipsia (BARLOW, 1959). The free water clearance is the most reliable way of assessing the effect of vasopressin.

In patients previously treated with vasopressin or posterior pituitary extracts, the presence of antibodies to vasopressin may obscure the results.

d) Depot-Pitressin Test

5 U of pitressin tannate in oil given intramuscularly leads to a decrease in the urinary output and to a rise in the urine concentration,

both with diabetes insipidus and with polydipsia. The patient with diabetes insipidus, however, feels relief at once and stops drinking. The polydipsic patient continues to drink, becomes overhydrated, gains weight, and develops headache, nausea, and confusion.

Method: 7 a.m. – after weighing the patient and measuring the hematocrit and the serum osmolality whenever possible – give 5 U depot-pitressin by i.m. injection. Fluid and food are not limited. Fluid intake, urinary output, and specific gravity are measured. Weight is measured 3 times daily. Serum osmolality and hematocrit daily. Patient is observed for at least 3 days.

e) Nicotine Test

α) Principle

Nicotine stimulates the release of vasopressin from the hypothalamic-neurohypophyseal system. According to DINGMAN, this stimulation does not occur via the osmoreceptors, but rather through direct action on the hypothalamic nuclei and the posterior pituitary. By means of an injection of nicotine, the content of vasopressin in the pituitary gland and its release can be examined. When the pituitary reacts to nicotine, urinary flow is again reduced due to the same mechanism as in the other tests.

β) Method

7:00 Empty bladder. Breakfast.

Between 7:00 and 7:30 fluid intake: 20 ml per kg body weight, i.e. 1.4 liters for a 70 kg patient. No coffee, no smoking.

Void urine quarter hourly. Drink same volume of fluid. Note the quarter hour urinary output and the specific gravity.

When urinary flow becomes constant, approximately at 10:00, at the beginning of a quarter hour period, 1 mg nicotine base is given intravenously to nonsmokers (1 mg nicotine base corresponds to 2 mg nicotine salicylate or 3 mg acidic nicotinetartrate).

Approximately 3 mg of nicotine base are injected into smokers.

The normal reaction to nicotine is the development of nausea. When this does not occur, the dose is too small and a slightly higher dose should be injected.

γ) Interpretation

It is assumed that two types of diabetes insipidus can be differentiated. If the patient does not react to nicotine, it must then be assumed that vasopressin is not formed. On the other hand, if the patient reacts to nicotine but not to osmotic stimulation, a lesion of the osmoreceptors must be assumed to be present (DINGMAN, 1957).

The stimulation threshold for the release of vasopressin by nicotine is very variable. Thus, a sufficiently high dose of nicotine cannot always be given to definitely establish a negative result (DIES, 1961).

f) Free Water Clearance

α) Principle

The urinary volume can be divided into two hypothetical portions, one with osmotically-bound water, and the other with free water. The urine volume per unit of time or the urinary flow is the sum of the osmolar clearance and the free water clearance ($V = C_{osm} + C_{H_2O}$). The latter quantity has a negative value in hypertonic urine and a positive value in hypotonic urine. The osmolar clearance is calculated from the formula:

$$C_{osm} = V \frac{\text{urine osm}}{\text{plasma osm}}$$

with V as the urinary output per minute. From this the formula for free water clearance is deduced

$$C_{H_2O} = V\left(1 - \frac{\text{urinary osm/kg } H_2O}{\text{plasma osm/kg } H_2O}\right).$$

Depending on the urinary and the plasma osmolality, the quotient will be >1 or <1, and the sum or the product will be negative or positive.

In general a negative free water clearance indicates that vasopressin is active. For convenience, the collection period for the estimation of the free water clearance is adjusted to the urinary flow. When the urinary flow is 5 ml/min, urine is collected every quarter hour. Hourly collections are made when the flow is <2 ml/min.

An exact estimation of the osmolality is essential for calculating the free water clearance. It allows exact calculation of diuresis, or antidiuresis, and it is advantageous to employ the free water clearance in the Hickey-Hare test, the nicotine test, and the vasopressin test. Thus, for example, a pathologic result may be identified as being due to simultaneous osmotic diuresis.

Where the urinary output is small, the clearance becomes inexact because of the unavoidable 5–10 ml of residual urine in the bladder.

β) Method

Patient hydrated, depending on the purpose of the investigation. When there is an average

urinary flow of 1–2 ml/min the urine is measured during 2 intervals, from 8–9 h and 9–10 h. The volume and the osmolality are measured.

Collection of blood at 9 h for estimation of the plasma osmolality. Use the formula above for the calculation.

In our experience other simplified tests such as comparison of the diuresis after drinking water or 1% saline or comparison of the urinary and plasma osmolarities during 6 to 12 h of fluid deprivation, are less reliable (JADRESIC, 1962; DECOURT, 1960; DASHE, 1963).

g) Assays of Plasma and Urinary Vasopressin

Until recently, the concentration of vasopressin in the body fluids and in tissue extracts could only be estimated from its antidiuretic and hypertensive effect in animals. Reliable results can be obtained using the unanesthetized trained dog (VAN DYKE, 1955), or by a less expensive method using the hydrated rat anesthetized with alcohol. The body fluid to be examined is injected intravenously (SAWYER, 1966). Diuresis may be registered by automatic counting of drops of urine (IRMSCHER, 1965), or a regular diuresis may be obtained by exposing the bladder and using a constant intravenous infusion (CZACZKES, 1964). It is possible to increase the sensitivity further by cannulating the abdominal aorta (SAWYER, 1966) or the jugular vein and by catheterizing the bladder (HEINTZ, 1964). 1–5 μU per ml are given as the lowest measurable values. A range of values between 0–0.05 to 22 μU/ml have been reported in the human, depending on the state of hydration (CZACZKES, 1964; LAUSON, 1967). Values of 2.5–5 μU/ml can be estimated by using brattleboro rats with hereditary diabetes insipidus which are more sensitive to vasopressin. In general, however, this sensitivity is hardly sufficient to determine the vasopressin concentration in human plasma, except in severe dehydration.

The biological estimation of vasopressin excreted in the urine has yielded values between 40–160 μU/24 h (CHAUDHURY, 1961). This method can only be conclusive in massive overproduction (see p. 63) (THORN, 1963). Radioimmunological methods for vasopressin are difficult to perform in biological fluids because of cross-reactions and interferences of unidentified substances with the hormone-antibody reaction. There are methods using antibodies produced by pure vasopressin (ROBERTSON, 1970) and others employing coupling of vasopressin to bovine serum albumin with glutaraldehyde. Previous gel filtration and acetone precipitation for elimination of the interfering substances are necessary for the determination of plasma vasopressin. Concentrations as low as 0.025 pg/ml or 0.01 μU can then be detected. In the healthy man, concentrations between 1–10 μμg/ml (= 0.1–1 μU) are found according to the state of hydration (ROBERTSON, 1970, 1971). In dehydrated diabetes insipidus patients concentrations of up to 0.6–3.2 pg/ml can be detected. In the unextracted urine rates of 1.2–6.5 mU/h arginine-vasopressin have been found in dehydration and rates of 0–0.35 mU/h during water loading. This material was dialyzable. The method is useful for the determination of secretory rates (OYAMA, 1971). The radioimmunological determination of neurophysin in plasma is easier to perform and the values parallel those of vasopressin corresponding to hydration and dehydration (CHENG, 1970; LEGROS, 1972). Further experience will show whether the determination of neurophysin can replace that of vasopressin.

10. Prognosis and Course

The course is irreversible almost without exception even when the cause of the symptomatic disturbance is successfully removed. Occasionally, diabetes insipidus can disappear after a craniopharyngeal cyst has been punctured. The prognosis of survival is good, providing a destructive process does not progress further. Spontaneous regression of symptoms is always suggestive of an additional lesion involving the anterior pituitary.

11. Therapy

In symptomatic diabetes insipidus, the underlying disorder must be treated primarily. The incidence of diabetes insipidus is high, when the etiology is unknown, and therefore irradiation of the pituitary region (in the hope

Table 7. Synopsis of investigation for diabetes insipidus

1. General examination, followed by ophthalmoscopy of the fundi, and urine examination for sugar and protein. Measurement of the urinary output and fluid intake with normal diet and unrestricted fluid intake. The specific gravity, and whenever possible, the osmolality of different urine samples are estimated.
2. Plasma and urinary osmolality in the morning in the fasting state or after 6 hours of absolute fluid deprivation.
3. Water deprivation test.
4. Intravenous vasopressin test, depot-pitressin test. Possibly test with hypertonic saline infusion and the nicotine test.
5. X-rays of the skull, antero-posterior and lateral views, possibly electroencephalography and pneumoencephalography.

of favorably influencing a possible neoplastic or granulomatous process) is not justified. Symptomatic treatment consists of vasopressin replacement, dietary measures, possibly salidiuretics and certain drugs.

Above all, the patient should be allowed to drink to satisfy his needs. The polyuria is decreased by reducing the intake of substances excreted in the urine, therefore a diet low in salt is advisable. The aqueous solution of a posterior pituitary extract and the synthetic lysin-vasopressin for i.m. administration have not proved satisfactory for replacement therapy. They are effective for only 4–6 hours, and when given in high doses produce side effects such as nausea, gastrointestinal spasms and diarrhea. On the other hand, pitressin-tannate in oil is very valuable for long-term treatment. Immediately before administration the ampoule must be warmed to body temperature and shaken well. An intramuscular injection of 1–3 units, equivalent to 0.2–0.6 ml, has a varying action lasting between 1 and 3 days. The patient is symptom-free during this period. Over-dosage easily leads to over-hydration, consequently the necessary dose must be individually adjusted. Occasionally a whole 1 ml ampoule, corresponding to 5 U, is needed. The full effect will be seen only after some days or weeks, when the high osmolality of the renal medulla is restored (HARRINGTON, 1968). Intelligent patients should be allowed to give the injection themselves as diabetics do. The use of pitressin in the form of snuff, although effective, is presently obsolete since it can give rise to the development of general or localized allergies and irritation of the nasal mucosa or even more serious complications, such as an allergic interstitial pneumonia, called "pituitary snuff-taker's lung" (PEPYS, 1966; BÜTIKOFER, 1970). On the other hand, aqueous solutions of synthetic lysin vasopressin are easily and conveniently employable in nasal sprays in a concentration of 50 μg/ml. Spraying must be undertaken every 3–6 hours, and the individually adequate dose must be determined. On an average, most patients need 50–62.5 U per day. When the duration of action does not last overnight, a cotton wool swab soaked in a vasopressin solution can be introduced. During a cold, buccal or vaginal administration is also possible. Spraying before retiring for the night can usually prevent nocturia, and urinary output usually falls to below 3 liters (DINGMAN, 1964). No effect on the uterus has been observed during pregnancy, and the blood pressure does not rise.

Synthetic vasopressin analogues such as DDAVP, 1-deamino-8-D-arginine-vasopressin, can have a higher antidiuretic potency together with a decreased pressor activity and a much longer half-life. Deamination in position 1 increases the antidiuretic effect, whereas the introduction of the D form of amino acid in position 8 is usually combined with a reduction of pressor effects. DDAVP (Desurin), administered intranasally with a simple and suitable device in doses of 15 μg, twice daily, normalizes the urine production in practically all idiopathic and symptomatic diabetes insipidus patients without any side effects. It will soon be commercially available and at present seems to be the safest and most convenient therapy (VAVRA, 1968; ANDERSON, 1972; IRMSCHER, 1972). A further indication might be Enuresis nocturna.

The paradoxical administration of thiazide diuretics is one way of decreasing the urinary volume in water diuresis (EARLEY, 1961). The effect is not satisfactorily explained, and is probably complex. Salidiuretics inhibit sodium reabsorption in the distal nephron and so decrease the formation of osmotically free water. In addition, thiazides are supposed to cause an increased proximal reabsorption of the isotonic filtrate, due to the excretion of sodium with diminished extracellular volume and restricted renal blood supply. This leads to decreased diuresis (EARLEY, 1962; CUTLER, 1962; ORLOFF, 1966). On the other hand, reduction of glomerular filtration rate with a reduced supply of isotonic solution to the distal tubule can hardly explain the beneficial effect of the salidiuretics. In patients with diabetes insipidus who respond well to depot-pitressin or the vasopressin spray, the addition of salidiuretics is of little benefit (CRAWFORD, 1960). In patients who have become resistant or over-sensitive to vasopressin, and especially in patients with nephrogenic diabetes insipidus, the complaints can be reduced and occasionally normalized through salt restriction and salidiuretics (chlorthalidone 200–400 mg daily, hydrochlorothiazide 50–75 mg), the potassium loss being compensated by daily doses of 3×1 g potassium salt. The slight antidiuretic action of aminopyrin and phenylbutazone are of no therapeutic significance now. The antidiabetic compound chlorpropamide in a dosage of 250–500 mg per day has an antidiuretic effect in patients with vasopressin-sensitive diabetes insipidus (ARDUINO, 1966; FRØYSHOV, 1968). The free water clearance becomes negative (HOCKEN, 1968; ANDREANI, 1969) by a mechanism of action which is still uncertain. Whether chlorpropamide acts by way of cyclic AMP (EARLEY, 1971) or not (INGELFINGER, 1969) is still undecided. Although a direct effect on the kidney is not entirely excluded (DANISI, 1970), most of the evidence is in favor of the view that chlorpropamide

enhances vasopressin's effect of increasing water permeability of the distal nephron (EARLEY, 1971; UHLICH, 1971). The most valid arguments are that chlorpropamide is ineffective in nephrogenic diabetes insipidus and in rats with hereditary absence of vasopressin but that it augments the effect of *small* doses of vasopressin (BERNDT, 1970). In severe cases of diabetes insipidus there is little or no effect. In a small percentage of patients with diabetes mellitus, it can lead to a reversible syndrome of inappropriate secretion of vasopressin with hyponatremia and hypo-osmolality (WEISSMANN, 1971). The hypoglycemic effect of chlorpropamide is a serious drawback for its use in patients with diabetes insipidus.

Whereas other peroral antidiabetic agents are ineffective, some other drugs, the anticonvulsant carbamazepine (Tegretol) and the anti-hypercholesteremic agent clofibrate (Atromide), have a similar antidiuretic action on patients with diabetes insipidus (BRAMHOFER, 1966; FRAHM, 1969; DECOURT, 1971). The urinary volume of patients with idiopathic or symptomatic diabetes insipidus can be reduced to half or less by peroral application of 2 or 3 doses of 200 mg carbamazepine. The side effects may be tiredness and dizziness, mainly at the beginning of the treatment. It is therefore advisable to start with small doses (100 mg 2 ×). Here again, the treatment is not or not completely effective in severe cases of diabetes insipidus. A combined treatment with smaller doses of chlorpropamide and carbamazepine (300 and 400 mg) may lessen or avoid the side-effects (STOLL, 1972). Clofibrate (Atromide), 4–6 × 0.25 can be combined with carbamazepine (PERLEMUTTER, 1971).

Not all patients want long-term replacement therapy. Many patients, especially those who developed the disorder during childhood, are accustomed to large fluid intake, have adjusted their lives accordingly, and no longer find it disturbing. They employ replacement therapy only in special situations when it is impossible to obtain the required volume of fluids.

F. Inappropriate Secretion of Antidiuretic Hormone (Schwartz-Bartter Syndrome, Oversecretion of Vasopressin)

1. Symptomatology and Pathogenesis

Hyponatremia with hypotonic hypervolemia and hypertonic urine has been observed in bronchial carcinoma and other diseases. In 1957, SCHWARTZ and BARTTER related these features to a dilution of the plasma due to hypersecretion of vasopressin. The diagnosis is made indirectly from the following findings:

1. Hyponatremia with hypo-osmolality of the serum and extracellular fluid.
2. Absence of hypovolemia and dehydration. Normal turgor, blood pressure, and serum urea.
3. Persistent natriuria despite hyponatremia. Hypertonic or not maximally diluted urine.
4. Intact renal and adrenal function.

Administration of exogenous vasopressin with simultaneous continued water intake results in increased glomerular filtration, elevated sodium excretion, hyponatremia, and dilution of the body fluids due to an increase of the plasma volume. Although the intravascular space is usually normal, the extracellular space becomes enlarged. Osmoregulation is abandoned in favor of maintenance of the volume of the body fluids. It is not clear why hypotonic hydration is well tolerated and the intracellular space does not enlarge. A shift of sodium into the intracellular space has been observed (KAYE, 1966) but it seems to be osmotically inactive. The elevated natriuresis is due to increased filtration and hypervolemia in the absence of aldosterone secretion, and also to disturbed sodium reabsorption in the presence of an enlarged extracellular volume (BARTTER, 1967); the additional effect of the hypothetic third factor (FICHMAN, 1968; BRICKER, 1967) may contribute to the natriuresis.

The syndrome has been observed:

1. in bronchial carcinoma, generally small-cell or anaplastic type. There has been only one reported case of inappropriate ADH with adenocarcinoma;
2. in cerebral diseases, primary and metastatic brain tumors, vascular diseases of the brain, trauma to the skull and malformations;
3. in certain metabolic disorders, such as porphyria with encephalopathy, and finally in an "idiopathic" group of unknown etiology (WALDVOGEL, 1967). It is not certain if this group also includes the syndrome observed in severe pulmonary tuberculosis, miliary tuberculosis, tuberculous meningitis and in the "cerebral salt-losing syndrome" (Table 8). Hyponatremia with hypovolemia usually occurs in Addison's disease, hypopituitarism and myxedema (see p. 159). There is currently some discussion as to whether hyponatremia, which occurs in postoperative conditions and in cardiac failure, is occasionally caused by excessive vasopressin secretion (BARTTER, 1967).

At first, there was only indirect support for an increased vasopressin secretion in these syndromes, as evidenced by a negative free water

Table 8. Occurrence of the vasopressin-oversecretion syndrome (inappropriate secretion of antidiuretic hormone) (BARTTER and SCHWARTZ, 1967)

1. Malignant tumors:	
	Carcinoma of the lung
	Duodenal carcinoma
	Pancreatic carcinoma
	Thymoma
2. Disorders of the central nervous system:	
	Meningitis
	Injuries to the skull
	Brain abscess
	Brain tumor
	Encephalitis
	Guillain-Barré syndrome
	Acute intermittent porphyria
	Subarachnoid hemorrhage
	Miscellaneous
3. Pulmonary diseases:	
	Pneumonias
	Tuberculosis
	Cavern formation (aspergillosis)
4. Idiopathic	

clearance. Moreover, the clearance may occasionally be positive, but it is always insufficiently high in relation to the hypotonic plasma. The kidneys are always able to retain sodium and to respond to mineralocorticoids, even with restricted renal blood supply.

Sodium administration increases natriuresis; therefore, hyponatremia can hardly be compensated by this means. On the other hand, when water deprivation restores the sodium concentration in the serum, hypernatriuria and polyuria are eliminated. Vasopression or substances with a vasopressin-like effect have been successfully demonstrated in the urine (THORN, 1963), in the plasma (BOWER, 1964), and in tumor extracts (ARMATRUDA, 1963; BOWER, 1964; BARRACLOUGH, 1966; BERDE, 1965, 1968; MARKS, 1968; ROBERTSON, 1971; UTIGER, 1966; VORHERR, 1968). However, an increased vasopressin concentration cannot be proven in all cases. It can be in the normal range, but the concentration is too high for the corresponding osmolality and is not suppressible by further hydration (BAUMANN, 1972).

Obviously there are two possible mechanisms:

1. Bronchial carcinomas or other tumors produce vasopressin or a similarly active substance. This is supported by the fact that the syndrome regresses after operation or after improvement in the pulmonary carcinoma following chemotherapy or radiation, and that such substances can be directly demonstrated in tumor extracts.

2. The pathogenesis of the syndrome in cerebral processes is probably related to a regulation disturbance in the release or production of vasopressin. The regulatory center no longer responds to the usual inhibitory stimuli (hypo-osmolality, hypervolemia, only partly to alcohol), but does, however, respond to diphenylhydantoin. This may allow a differential diagnosis between the syndrome caused by tumor and the one caused by central nervous disorders. It is not known whether and how cases of the Schwartz-Bartter syndrome occuring in pulmonary tuberculosis, are to be included here. The hyponatremia in myxedema and in pituitary insufficiency does not appear to belong to this syndrome (see p. 159, 95).

2. Differential Diagnosis and Diagnosis

The hyponatremia due to "true" salt depletion, such as occurs in ADDISON's disease, in tubular nephropathy, and during vomiting and diarrhea, lead to *hypo*volemia, hypo-osmolality, hypotension, hypotonic dehydration and azotemia. Hyponatremia and *hyper*osmolality are suggestive of mannitol abuse (AVIRAN, 1967). Hypertonic urine can also be produced when the glomerular filtration is reduced. This, however, is always associated with azotemia and an increase in plasma creatinine. Moreover, sodium excretion decreases with a fall in filtration.

The hyponatremia in terminal cardiac failure is combined with severe edema. Salt escapes into the interstitial space and into the tissue, sodium excretion is reduced, and the sodium balance is positive.

The hyponatremia in hypopituitarism (see p. 95) is not connected with hypernatriuria and does not improve with dehydration, but does respond immediately to cortisone treatment. Psychogenic or organic polydipsia can give rise to similar findings when the fluid intake is more than 25 ml/min, although the urine is always maximally diluted.

Pseudohyponatremia in hyperlipemia and in hyperproteinemia can be excluded because of the normal osmolality.

Finally, administration of mannitol can be a cause of hyponatremia with hyperosmolality of the serum (AVIRAM, 1967). The Schwartz-Bartter syndrome is present when after exclusion of dehydration and cardiac failure, hyponatremia occurs with hypertonic or insufficiently diluted urine and can be improved by limiting the fluid intake. Values of the urea in the serum are charcteristically very low, being under 20 mg%.

3. Course and Therapy

The course is determined by the primary disease. Remissions are possible, especially in porphyria. When the basic disease cannot be influenced, the hyponatremia can be improved or be avoided by limiting water intake. The general condition of the patient is thus improved.

Diphenylhydantoin which i.v. inhibits vasopressin overproduction in the central nervous system does not correct long-standing hyponatremia (FICHMAN, 1968). Although the application of mannitol counteracts the effects of vasopressin oversecretion, it has no advantage over fluid restriction alone.

References

Reviews

BERDE, B.: Recent progress in oxytoxin research. Springfield: Thomas 1959.

— Neurohypophysial hormones and similar polypeptides. In: Handbuch der experimentellen Pharmakologie, vol. XXII. Berlin-Heidelberg-New York: Springer 1968.

KLEEMAN, C.R., CUTLER, R.E.: The neurohypophysis. Ann. Rev. Physiol. **25**, 385 (1963).

SACHS, H., FAWCETT, P., TAKABATAKE, Y., PORTANOVA, R.: Biosynthesis and release of vasopressin and neurophysin. Recent Progr. Hormone Res. **25**, 447 (1969).

SCHWARTZ, I.L., SCHWARTZ, W.B.: Symposium on antidiuretic hormones. Amer. J. Med. **42**, 651 (1967).

STUTINSKY, F.: Neurosecretion. Berlin-Heidelberg-New York: Springer 1967.

WALKER, J.M.: Vasopressin. In: GRAY, C.H., and A.L. BACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

WOLSTENHOLME, G.E.W., BIRCH, J. (eds.): Neurohypophysial hormones. Study Group No 39. Ciba Foundation Volumes, Livingstone: Churchill 1971.

Embryology and Anatomy (see Chap. II and V)

Biochemistry

ACHER, R.: Chemistry of neurohypophysial hormones. In: HARRIS and DONOVAN, The pituitary gland, vol. III. London: Butterworths 1966.

— Evolution des hormones neurohypophysaires. In: GUAL, Progress in endocrinology. Amsterdam: Excerpta medica foundation 1969.

BERDE, B., BOISSONNAS, R.A.: Synthetic analogues and homologues of the posterior pituitary hormones. In: HARRIS and DONOVAN, The pituitary gland, vol. III. London: Butterworths 1966.

— — Basic pharmacological properties of synthetic analogues and homologues of the neurohypophysical hormones. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.

— — HUGUENIN, R.L., STÜRMER, E.: Vasopressin analogues with selective pressor activity. Experientia (Basel) **20**, 42 (1964).

— CERLETTI, A.: Über die antidiuretische Wirkung von synthetischem Lysin-Vasopressin. Helv. physiol. pharmacol. Acta **19**, 135 (1961).

BOISSONNAS, R.A., GUTTMANN, S.T.: Chemistry of the neurohypophysial hormones. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.

— — BERDE, B, KONZETT, H.: Relationships between the chemical structures and the biological properties of the posterior pituitary hormones and their synthetic analogues. Experientia (Basel) **77**, 377 (1961).

GUHL, U.: Die antidiuretische und pressorische Wirksamkeit von Arginin^8-Vasopressin, Lysin^8-Vasopressin und Phenylalanin^2-lysin^8-Vasopressin beim Menschen. Schweiz. med. Wschr. **91**, 798 (1961).

LIGHT, A., VIGNEAUD, V. DU: On the nature of oxytocin and vasopressin from human pituitary. Proc. Soc. exp. Biol. (N.Y.) **98**, 692 (1958).

SAWYER, W.H.: Evolution of antidiuretic hormones and their functions. Amer. J. Med. **42**, 678 (1967).

TSAKIRIS, A., HAEMMERLI, U.P., BÜHLMANN, A.: Reduction of portal venous pressure in cirrhotic patients with bleeding from oesophageal varices, by administration of a vasopressin derivative, phenylalanine^2-lysine^8-vasopressin. Amer. J. Med. **36**, 825 (1964).

VIGNEAUD, V. DU, GISH, D.T., KATSOYANNIS, P.G.: A synthetic preparation possessing biological properties, associated with arginine-vasopressin. J. Amer. chem. Soc. **76**, 475 (1954).

VLIEGENTHART, J.F.G., VERSTEEG, D.H.G.: The evolution of the vertebrate neurohypophysial hormones in relation to the genetic code. J. Endocr. **38**, 3 (1967).

WALTER, R., RUDINGER, J., SCHWARTZ, I.L.: Chemistry and structure-activity relations of the antidiuretic hormones. Amer. J. Med. **42**, 653 (1967).

WOOLEY, D.W., MERRIFIELD, R.B.: Specificity of peptides. Science **128**, 238 (1958).

Transport, Metabolism, Excretion

CHENG, K.W., FRIESEN, H.G.: Physiologic factors regulating the secretion of neurophysin. Metabolism **19**, 876 (1970).

CZACZKES, J.W., KLEEMAN, C.R., KOENIG, M.: Physiologic studies of antidiuretic hormone by its direct measurement in human plasma. J. clin. Invest. **43**, 1625 (1964).

DICKER, S.E.: Release and metabolism of neuro-hypophyseal hormones. J. Pharm. pharmacol. **13**, 449 (1961).

GINSBURG, M.: Production, release, transportation and elimination of the neurohypophysial hormones. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.

— JAYASENA, K., THOMAS, P.J.: The preparation and properties of porcine neurophysin and the influence of calcium on the hormone-neurophysin complex. J. Physiol. (Lond.) **184**, 387 (1966).

HELLER, H., GINSBURG, M.: Secretion, metabolism and fate of the posterior pituitary hormones. In: HARRIS and DONOVAN, The pituitary gland, vol. III. London: Butterworths 1966.

LAUSON, H.D.: Vasopressin and oxytocin in the plasma of man and other mammals. In: ANTONIADES, H., Hormones in human plasma. Boston: Little Brown & Co. 1960.

— Metabolism of antidiuretic hormones. Amer. J. Med. **42**, 713 (1967).

— BOCANEGRA, M.: Clearance of exogenous vasopressin from plasma of dogs. Amer. J. Physiol. **200**, 493 (1961).

LEGROS, J.J., FRANCHIMONT, P.: Human neurophysine blood levels under normal, experimental and pathological conditions. Clin. Endocr. **1**, 99 (1972).

SILVER, L., SCHWARTZ, I.L., FONG, C.T.O., DEBONS, A.F., DAHL, L.K.: Disappearance of plasma radioactivity after injection of H^3- or I^{131}-labelled arginine vasopressin. J. appl. Physiol. **16**, 1097 (1961).

THORN, N. A.: Some chemical properties of antidiuretic material in the urine of rats. Acta endocr. (Kbh.) **32**, 128 (1959).

Localization of Synthesis

BARGMANN, W.: Neurohypophysis. Structure and function. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.

HELLER, H.: The hormone content of the vertebrate hypothalamo-neurohypophysial system. Brit. med. Bull. **22**, 227 (1966).

INGRAM, W. R., FISHER, C., RANSON, S. W.: Experimental diabetes insipidus in the monkey. Arch. intern. Med. **57**, 1067 (1936).

JEWELL, P. A., VERNEY, E. B.: An experimental attempt to determine the site of the neurohypophysial osmoreceptors in the dog. Phil. Trans **240**, 197 (1956/57).

LEDERIS, K.: The distribution of vasopressin and oxytocin in hypothalamic nuclei. In: 3rd Internat. Symposium on Neurosecretion, ed. by H. HELLER and R. B. CLARK. New York: Academic Press 1962.

OLIVECRONA, H.: Paraventricular nucleus and pituitary gland. Acta physiol. scand. **40**, Suppl. 136 (1957).

SACHS, H.: Biosynthesis and release of vasopressin. Amer. J. Med. **42**, 687 (1967).

SCHARRER, E., SCHARRER, B.: Neurosekretion. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. VI-V. Berlin-Göttingen-Heidelberg: Springer 1954.

Release

ARNDT, J. O.: Diuresis reduced by water infusion into the carotid loops and its inhibition by small hemorrhage. The competition of volume- and osmocontrol. Pflügers Arch. ges. Physiol. **282**, 313 (1965).

AUBRY, R. H., NANKIN, H. R., MOSES, A. M., STREETEN, D. H. P.: Measurement of the osmotic threshold for vasopressin release in human subjects, and its modification by cortisol. J. clin. Endocr. **25**, 1481 (1965).

DICKER, S. E.: La secrétion des hormones neurohypophysaires chez l'adulte et le nouveau-né. In: H. P. KLOTZ, Symposium international sur la neuroendocrinologie. Paris: Expansion sci. frc. 1966.

DINGMAN, J. F.: Hypothalamus and the endocrine control of sodium and water metabolism. Am. J. med. Sci. **235**, 79 (1958).

— BENIRSCHKE, K., THORN, G. W.: Studies of neurohypophyseal function in man. Amer. J. Med. **23**, 226 (1957).

FICHMAN, M. P., BETHUNE, J. E., KLEEMAN, C. R.: Inhibition of antidiuretic hormone by diphenylhydantoin. Clin. Res. **15**, 141 (1967).

FRIBERG, O.: The antidiuretic effect of coitus in human subjects. Acta endocr. (Kbh.) **12**, 193 (1953).

GAUER, O. H., HENRY, J. P.: Circulatory basis of fluid volume control. Physiol. Rev. **43**, 423 (1963).

HARRIS, G. W., PICKLER, v. R.: Reflex stimulation of the neurohypophysis (posterior pituitary gland) and the nature of posterior pituitary hormone(s). Nature (Lond.) **172**, 1049 (1953).

HOFER, M. A., HINKLE, L. E., JR.: Production of conditioned diuresis in man. J. clin. Invest. **42**, 1421 (1963).

HULET, W. H., SHAPIRO, T., SCHWARTZ, B. E., SMITH, H. W.: Water diuresis after hypnotic suggestion in hydropenic subjects. J. appl. Physiol. **18**, 186 (1963).

MOSES, A. M., MILLER, M., STREETEN, D. H. P.: Quantitative influence of blood volume expansion on the osmotic threshold for vasopressin release. J. clin. Endocr. **27**, 655 (1967).

PITTS, R. F.: Physiology of the kidney and body fluids, 2nd ed. Chicago: Year-book Medical Publishers 1968.

SACHS, H.: Biosynthesis and release of Vasopressin. J. Amer. Med. **42**, 687 (1967).

— FAWCETT, P., TAKABATAKE, Y., PORTANOVA, R.: Biosynthesis and release of vasopressin and neurophysin. Recent Progr. Hormone Res. **25**, 447 (1969).

SAWYER, W. H., MILLS, E.: Control of Vasopressin secretion. In: MARTINI, L., and W. F. GANONG, Neuroendocrinology. New York: Academic Press 1966.

SHARE, L.: Vasopressin, its bioassay and the physiological control of its release. Amer. J. Med. **42**, 701 (1967).

THORN, N. A.: Role of calcium in the release of vasopressin and oxytocin from posterior pituitary protein. Acta endocr. (Kbh.) **50**, 357 (1965).

— Anti-diuretic hormone synthesis. Release and action under normal and pathological circumstances. Adv. metabol. Disorders **4**, 39 (1970).

Regulation of Water Uptake—Thirst Center

ANDERSSON, B.: Polydipsia, antidiuresis and milk ejection caused by hypothalamic stimulation. In: HELLER, The Neurohypophysis. London: Butterworth's 1957.

— MCCANN, S. M.: Hypothalamic control of water intake. J. Physiol. (Lond.) **129**, 442 (1955).

FITZSIMONS, J. T.: The hypothalamus and drinking. Brit. med. Bull. **22**, 232 (1966).

— Thirst. Physiol. Rev. **52**, 468 (1972).

GREER, M. A.: Suggestive evidence of a primary "drinking center" in hypothalamus of the rat. Proc. Soc. exp. Biol. (N.Y.) **89**, 59 (1955).

STEVENSON, J. A. F., WELT, L. G., ORLOFF, J.: Abnormalities of water and electrolyte metabolism in rats with hypothalamic lesions. Amer. J. Physiol. **161**, 35 (1950).

STRAUSS, M. P.: Thirst—the acquisition of water. Arch. intern. Med. **101**, 216 (1958).

WAYNER, M. J.: Thirst. Proc. of the first internat. Symp. on thirst in the regulation of body water. Oxford: Pergamon Press 1964.

Effect of Oxytocin

FITZPATRICK, R. J.: The neurohypophysis and the male reproductive tract. In: HARRIS and DONOVAN, The piuitary gland, vol. III. London: Butterworths 1966.

MIRSKY, I. A., PERISUTTI, G.: Action of oxytocin and related peptides on epididymal adipose tissue of the rat. Endocrinology **71**, 158 (1962).

RUDINGER, J., PLIŠKA, V., KREJČÍ, I.: Oxytocin analogs in the analysis of some phases of hormone action. Recent Progr. Hormone Res. **28**, 131 (1972).

Effect of Vasopressin on Diuresis

BARATZ, R. A., DOIG, A., ADATTO, I. J.: Plasma antidiuretic activity and free water clearance following osmoreceptor and neurohypophyseal stimulation in human subjects. J. clin. Invest. **39**, 1539 (1960).

BERLINER, R. W., BENETT, C. M.: Concentration of urine in the mammalian kidney. Amer. J. Med. **42**, 777 (1967).

BRUNNER, F., RECTOR, F. C., JR., SELDIN, D. W.: Mechanism of glomerulotubular balance. II. Regulation of proximal tubular reabsorption by tubular volume as studied by stopped-flow microperfusion. J. clin. Invest. **45**, 603 (1966).

BUCHBORN, E.: Zur Endokrinologie des distalen Nephron. II. Mitteil. ADH-Plasmaspiegel und konzentrative Nierenleistungen. Klin. Wschr. **39**, 17 (1961).
— Klinische Pathophysiologie der Harnkonzentrierung. Schweiz. med. Wschr. **94**, 1273 (1964).
— ANASTASAKIS, S., EDEL, H., EIGLER, J., RENNER, E.: Die osmotische Diurese in der Beurteilung und Differenzierung von Störungen der Harnkonzentrierung. Verh. Dtsch. Ges. inn. Med. 69. Kongr., S. 702 (1963).
CAPEK, C., RUMRICH, G., ULBRICH, K. J.: Permeabilität der corticalen Tubulusabschnitte von Ratten für Harnstoff. Pflügers Arch. ges. Physiol. **283**, 1224 (1965).
DI BONA, D. R., CIVAN, M. M., LEAF, A.: The cellular specificity of the effect of vasopressin on toad urinary bladder. J. Membrane Biol. **1**, 79 (1969).
GARDNER, K. D., JR., MATTEY, R. H.: An in vitro demonstration of increased collecting tubular permeability to urea in the presence of vasopressin. J. clin. Invest. **43**, 1968 (1964).
GOTTSCHALK, C. W.: Osmotic concentration and dilution of the urine. Amer. J. Med. **36**, 670 (1964).
HARRINGTON, A. R., VALENTIN, H.: Impaired urinary concentration after vasopressin and its gradual correction in hypothalamic diabetes insipidus. J. clin. Invest. **47**, 502 (1968).
HAYS, R. M., LEAF, A.: Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. J. gen. Physiol. **45**, 905 (1962).
JAENIKE, J. R.: The influence of vasopressin on the permeability of the mammalian collecting duct to urea. J. clin. Invest. **40**, 144 (1961).
— The relative rates of urea and water permeation in the distal nephron of the intact kidney. J. clin. Invest. **43**, 45 (1964).
KERRIGAN, G. A., TALBOT, N. B., CRAWFORD, J. D.: Role of the neurohypophyseal-antidiuretic-hormone-renal system in everyday clinical medicine. J. clin. Endocr. **15**, 265 (1955).
KLEEMAN, C. R., CZAZKES, J. W., CUTLER, R.: Mechanism of impaired water excretion in adrenal and pituitary insufficiency. IV. Antidiuretic hormone in primary and secondary adrenal insufficiency. J. clin. Invest. **43**, 1641 (1964).
— FICHMAN, M. P.: The clinical physiology of water metabolism. New Engl. J. Med. **277**, 1300 (1967).
KRAMER, K., THURAU, K., DEETJEN, P.: Hämodynamik des Nierenmarks. Pflügers Arch. ges. Physiol. **270**, 251 (1960).
LABHART, A.: Niere bei zentralem Diabetes insipidus. In: Handbuch der inneren Medizin, Bd. VIII Niere, hrsg. von H. SCHWIEGK. Berlin-Heidelberg-New York: Springer 1967.
LEAF, A.: Kidney water and electrolytes. Ann. Rev. Physiol. **22**, 111 (1960).
— MAMBY, A. R.: An antidiuretic mechanism not regulated by extracellular fluid tonicity. J. clin. Invest. **31**, 60 (1952).
O'DELL, R., SCHMIDT-NIELSEN, B.: Concentrating ability and kidney structure. Fed. Proc. **19**, 366 (1960).
PICKFORD, M.: Neurohypophysis and kidney function. In: The pituitary gland, vol. III, ed. HARRIS and DONOVAN. London: Butterworths 1966.
SCHMIDT-NIELSEN, B.: Urea excretion in mammals. Physiol. Rev. **38**, 139 (1958).
SCHOLANDER, P. F.: The wonderful net. Sci. Amer. **196**, 96 (1957).
SMITH, H. W.: The kidney. New York: Oxford University Press 1951.
SOLOMON, A. K.: Pumps in the living cell. Sci. Amer. **207**, 100 (1962).
TALBOT, N. B., SOBEL, E. H., MCARTHUR, J. W., CRAWFORD, J. D.: Functional endocrinology. Cambridge: Harvard University Press 1952.
THORN, N. A.: The influence of the neurohypophysial hormones and similar polypeptids on the kidneys. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.
THURAU, K., DEETJEN, P., KRAMER, K.: Hämodynamik des Nierenmarkes. Wechselbeziehungen zwischen vaskulärem und tubulärem Gegenstromsystem bei arterieller Drucksteigerung, Wasserdiurese und osmotischer Diurese. Pflügers Arch. ges. Physiol. **279**, 270 (1960).
ULLRICH, K. J., KRAMER, K., BOYLAN, J. W.: Mechanism of urinary concentration. In: D. A. K. BLACK, Renal disease. Oxford: Blackwell 1962.
VERNEY, E. B.: Absorption and excretion of water. The antidiuretic hormone. Lancet **1946II**, 739, 781.
WARDENER, H. E. DE: Polyuria. In: BLACK, D. A. K., Renal disease. Oxford: Blackwell 1962.
WIRZ, H., in: The location of antidiuretic action in the mammalian kidney. In: HELLER, Neurohypophysis. London: Butterworths 1957.
— Kidney, water and electrolyte metabolism. Ann. Rev. Physiol. **23**, 577 (1961).

Influences on the Antidiuretic Vasopressin Effect

BANK, N., AYNEDJIAN, H. S.: A micropuncture study of the renal concentrating defect of potassium depletion. Amer. J. Physiol. **206**, 1347 (1964).
— — On the mechanism of hyposthenuria in hypercalcemia. J. clin. Invest. **44**, 681 (1965).
BRUNNER, F. P., RECTOR, F. C., JR., SELDIN, D. W.: The mechanism of the urinary concentrating defect in potassium deficient rats. Pflügers Arch. ges. Physiol. **290**, 202 (1966).
CARONE, F. A., EPSTEIN, F. H., BECK, D., LEVITIN, H.: The effects upon the kidney of transient hypercalcemia induced by parathyroid extract. Amer. J. Path. **36**, 77 (1960).
DINGMAN, J. F., DESPOINTES, R.: Adrenal steroid inhibition of vasopressin release from neurohypophysis of normal subjects and patients with Addison's disease. J. clin. Invest. **39**, 1851 (1960).
EPSTEIN, F. H., BECK, D., CARONE, F. A., LEVITIN, H., MANITIUS, A.: Changes in renal concentrating ability produced by parathyroid extract. J. clin. Invest. **38**, 1214 (1959).
— KLEEMAN, C. R., HENDRIKS, A.: The influence of bodily hydration on the renal concentrating process. J. clin. Invest. **36**, 629 (1957).
HAYS, R. M., LEAF, A.: The problem of clinical vasopressin-resistance: in vitro studies. Ann. intern. Med. **54**, 700 (1961).
KLEEMAN, C. R., MAXWELL, M. H., WITLIN, S.: Functional isothenuria, an isolated reversible renal tubular defect. Arch. intern. Med. **101**, 1023 (1958).
LEVITIN, H., GOODMAN, A., PIGEON, G., EPSTEIN, F. H.: Composition of the renal medulla during water diuresis. J. clin. Invest. **41**, 1145 (1962).
MANITIUS, A., LEVITIN, H., BECK, D., EPSTEIN, F. H.: On the mechanism of impairment of renal concentrating ability in hypercalcemia. J. clin. Invest. **39**, 193 (1960).
— — — — On the mechanism of impairment of renal concentrating ability in potassium deficiency. J. clin. Invest. **39**, 684 (1960).
RELMAN, A. S., SCHWARTZ, W. B.: The nephropathy of potassium depletion. New Engl. J. Med. **255**, 195 (1956).
— — The kidney in potassium depletion. Amer. J. med. **24**, 764 (1958).
ULLMAN, T. D., CZACZKES, W. J., MENCZEL, J.: Modification of the antidiuretic effect of vasopressin by acid and alcaline loads. J. clin. Invest. **44**, 754 (1965).

ULLRICH, K. J., RUMRICH, G., FUCHS, G.: Wasserpermeabilität und transtubulärer Wasserfluß corticaler Nephronabschnitte bei verschiedenen Diuresezuständen. Pflügers Arch. ges. Physiol. **280**, 99 (1964).

Antidiuretic Mode of Action of Vasopressin

BERLYNE, G. M.: Urinary hyaluronidase. Nature (Lond.) **185**, 389 (1960).

DARMADY, E. M., DURANT, J., MATTHEWS, E. R., STRANACK, F.: Location of ^{131}I Pitressin in the kidney by autoradiography. Clin. Sci. **19**, 229 (1960).

FONG, C. T. O., SILVER, L., CHRISTMAN, D. R., SCHWARTZ, I. L.: On the mechanism of action of the antidiuretic hormone (vasopressin). Proc. nat. Acad. Sci. (Wash.) **46**, 1273 (1960).

GINETZINSKY, A. G.: Role of hyaluronidase in the reabsorption of water in renal tubules: The mechanism of action of the antidiuretic hormone. Nature (Lond.) **182**, 1218 (1958).

GRANTHAM, J. J., BURG, M. B.: Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubulus. Amer. J. Physiol. **211**, 255 (1966).

LEAF, A.: Transepithelial transport and its hormonal control in toad bladder. Ergebn. Physiol. **56**, 216 (1965).

— Membrane effects of antidiuretic hormone. Amer. J. Med. **42**, 745 (1967).

ORLOFF, J., HANDLER, J. S.: The similarity of effects of vasopressin, adenosine-3′,5′-phosphate (cyclic AMP) and theophylline on the toad bladder. J. clin. Invest. **41**, 702 (1962).

— — The cellular mode of action of antidiuretic hormone. Amer. J. Med. **36**, 686 (1964).

— — The role of Adenosin-3′-,5′-Phosphate in the action of antidiuretic hormone. Amer. J. Med. **42**, 757 (1967).

RASMUSSEN, H., SCHWARTZ, I. L., SCHOESSLER, M. A., HOCHSTER, G.: Studies on the mechanism of action of vasopressin. Proc. nat. Acad. Sci. (Wash.) **46**, 1278 (1960).

SCHWARTZ, I. L., LIVINGSTONE, L. M.: Cellular and molecular aspects of the antidiuretic action of vasopressins and related peptides. Vitam. and Horm. **22**, 261 (1964).

— RASMUSSEN, H., SCHOESSLER, M. A., SILVER, L., FONG, C. T. O.: Relation of chemical attachement to physiological action of vasopressin. Proc. nat. Acad. Sci. (Wash.) **46**, 1288 (1960).

— WALTER, R.: Factors influencing the reactivity of the toad bladder to the hydro-osmotic action of vasopressin. Amer. J. Med. **42**, 769 (1967).

SHARP, G. W. G., LEAF, A.: Mechanism of action of Aldosterone. Physiol. Rev. **46**, 593 (1966).

Other and Questionable Physiological Effects of Vasopressin

BALASSE, E., RASIO, E., CONARD, V.: Action insulino-mimétique de la vasopressine chez le chien. Arch. int. Pharmacodyn. **161**, 392 (1966).

BERGEN, ST. S., JR., SULLIVAN, R., HILTON, J. G., WILLIS, S. W., JR., ITALLIE, TH. B. VAN: Glycogenolytic effect of vasopressin in the canine liver. Amer. J. Physiol. **199**, 136 (1960).

HILTON, G., SCIAN, L. F., WESTERMANN, C. D., NAKANO, J., KRUESI, O. R.: Vasopressin stimulation of the isolated adrenal glands: nature and mechanism of hydrocortisone secretion. Endocrinology **67**, 298 (1960).

MIRSKY, I. A.: Effect of oxytocin, vasopressin and related peptides on plasma free fatty acids. Amer. J. Physiol. **204**, 842 (1963).

PASQUALINI, R. Q., CODEVILLA, A.: Thirst suppressing ("antidipsetic") effect of pitressin in diabetes insipidus. Acta endocr. (Kbh.) **30**, 37 (1959).

PICKFORD, M.: Neurohypophysis and vascular reactions in HARRIS and DONOVAN. The pituitary gland, vol. III. London: Butterworths.

SOERGEL, K. H., WHALEN, G. E., HARRIS, J. A., GREENEN, J. E.: Effect of antidiuretic hormone on human small intestinal water and solute transport. J. clin. Invest. **47**, 1071 (1968).

Etiology and Clinical Aspects of Diabetes Insipidus

ANGST, J.: Die Psychiatrie des Diabetes insipidus. Arch. Psychiat. Nervenkr. **199**, 663 (1959).

AUGILÒ, F., VEGA, L. A., HADDOCK, L., RODRIGUEZ, O.: Diabetes insipidus syndrome in hypopituitarism of pregnancy. Acta endocr. (Kbh.), Suppl. 137 zu Vol. 60 (1969).

BAGNAL, A. G., MCIVER, C., FORD, D. K., PALMER, R. A.: Alterations in the state of diabetes insipidus resulting from co-existent hypofunction of the anterior pituitary and adrenal cortex: studies on a case of diffuse meningiomatosis. Amer. J. Med. **17**, 115 (1954).

BLEULER, M.: Endokrinologische Psychiatrie. Stuttgart: Thieme 1954.

BLOTNER, H.: Diabetes insipidus. New York: Oxford University Press 1951.

— Primary or idiopathic diabetes insipidus: a system disease. Metabolism **7**, 191 (1958).

COGGINS, C. H., LEAF, A.: Diabetes insipidus. Amer. J. Med. **42**, 807 (1967).

DUBACH, V. C., WIESLI, B.: Histiocytosis „X" mit Diabetes insipidus, Vulvaulcerationen, Spontanpneumothorax, Hypothyreose und Exitus an Urämie. Schweiz. med. Wschr. **98**, 117 (1968).

EGLIN, J. M., JR., JESSIMAN, A. G.: Inactivation of the antidiuretic activity of vasopressin during pregnancy: A report of a case. J. clin. Endocr. **19**, 369 (1959).

FANCONI, G.: Zur Differentialdiagnose des Diabetes insipidus. Helv. paediat. Acta **11**, 506 (1956).

FINK, E. B.: Diabetes insipidus, a clinical review and analysis of necropsy reports. Arch. Path. **6**, 102 (1928).

HERRMANN, E.: Maskierter Diabetes insipidus bei Ausfall der Praehypophyse. Schweiz. med. Wschr. **1955**, 1041.

IRMSCHER, K.: Beiträge zur Klinik des Diabetes insipidus mit Untersuchungen über die Adiuretin-Konzentration im Blutplasma des Menschen. Habilitationsschrift, Düsseldorf 1967.

KOURILSKY, R.: Le diabète insipide humain. Ann. Méd. **48**, 288 (1947).

— Diabetes insipidus. Proc. roy. Soc. Med. **43**, 842 (1950).

LIEBERMAN, PH. H., JONES, CH. R., DARGION, H. W. K., BEGG, CH. F.: A reappraisel of eosinophilic granuloma of bone, Hand-Schüller-Christian syndrom and Letterer-Siewe syndrom. Medicine (Baltimore) **48**, 375 (1969).

LÜTHY, F., KLINGER, M.: Der Tumoretten-Tumor des Hypophysenhinterlappens. Schweiz. Z. Path. **14**, 721 (1951).

MARTIN, M. M.: Combined anterior pituitary and neurohypophyseal insufficiency. Studies of body fluid spaces and renal function. J. clin. Invest. **38**, 882 (1959).

SCHEER, R. L., RAISE, L. G., LLOYD, CH. W.: Changes in diabetes insipidus during pregnancy and lactation. J. clin. Endocr. **19**, 805 (1959).

SLOPER, J. C., KARIM, M. A., RICHARDS, M. A.: Pathological aspects of the concept of neurosecretion with special references to the pathogenesis of Diabetes insipidus. In: STUTINSKY, F., Neurosecretion. Berlin-Heidelberg-New York: Springer 1967.

SMITH, H. W.: The kidney. New York: Oxford University Press 1951.

THOMAS, W. C., JR.: Diabetes insipidus. J. clin. Endocr. **17**, 565 (1957).

UHLICH, E., BUCHBORN, E.: Diabetes insipidus. Internist (Berl.) **13**, 141 (1972).

Diabetes Insipidus after Hypophysectomy or Section of the Pituitary Stalk

IKKOS, D., LUFT, R., OLIVECRONA, H.: Hypophysectomy in man: Effect on water excretion during the first two postoperative months. J. clin. Endocr. **15**, 553 (1955).
KOVACS, K., DAVID, M. A., LASZLO, F. A.: Adrenocortical function in rats after lesion of the pituitary stalk. J. Endocr. **25**, 9 (1962).
LIPSETT, M. B., MACLEAN, J. P., WEST, D. C., LI, M. C., PEARSON, O. H.: An analysis of the polyuria induced by hypophysectomy in man. J. clin. Endocr. **16**, 183 (1956).
RANDALL, R. V., CLARK, E. C., DODGE, H. W., JR., LOVE, J. G.: Polyuria after operation for tumors in the region of the hypophysis and hypothalamus. J. clin. Endocr. **20**, 1614 (1960).
SHARKEY, P. C., PERRY, J. H., EHNI, G.: Diabetes insipidus following section of hypophyseal stalk. J. Neurosurg. **18**, 445 (1961).

Hereditary Central Diabetes Insipidus

BRAVERMAN, L. W., MANCINI, J. P., MCGOLDRICK, D. M.: Hereditary idiopathic diabetes insipidus. A case report with autopsy findings. Ann. intern. Med. **63**, 503 (1965).
FORSSMAN, H.: Two different mutations of the *x*-Chromosome causing diabetes insipidus. Amer. J. hum. Genet. **7**, 21 (1955).
GREEN, J. R., BUCHAN, G. C., ALOORD, E. C., JR., SWANSON, A. G.: Hereditary idiopathic types of diabetes insipidus. Clin. Res. **14**, 131 (1966).
HANHART, E.: Die Erbpathologie des Diabetes insipidus. In: Handbuch der Erbpathologie, Bd. 4. Berlin: Springer 1940.
LEVINGER, E. L., ESCAMILLA, R. F.: Hereditary diabetes insipidus: report of 20 cases in 7 generations. J. clin. Endocr. **15**, 547 (1955).
MARTIN, F. I. R.: Familial diabetes insipidus. Quart. J. Med. **28**, 573 (1959).
VALTIN, H.: Hereditary hypothalamic diabetes insipidus in rats (Brattleboro strain). A useful experimental model. Amer. J. Med. **42**, 814 (1967).
WEIL, A., JR.: Über die hereditäre Form des Diabetes insipidus. Dtsch. Arch. klin. Med. **93**, 180 (1908).

Nephrogenic Hereditary Diabetes Insipidus

BODE, H. H., CRAWFORD, J. D.: Nephrogenic diabetes insipidus in North America—The Hopewell hypothesis. New Engl. J. Med. **280**, 750 (1969).
CARTER, C., SIMPKISS, M.: The carrier state in nephrogenic diabetes insipidus. Lancet **1956II**, 1069.
CUTLER, R. E., KLEEMAN, C. R., MAXWELL, M. H., DOWLING, J. T.: Physiologic studies in nephrogenic diabetes insipidus. J. clin. Endocr. **22**, 827 (1962),
FORSSMAN, H.: Form of diabetes insipidus characterized by sex-linked inheritance and unresponsiveness to the antidiuretic hormone, new genotypic entity. Acta endocr. (Kbh.) **16**, 355 (1954).
GAUTIER, A., PRADER, A.: Un cas de diabète insipide néphrogène chez un nourrisson avec absence initiale de soif („diabète insipide occulte"). Helv. paediat. Acta **11**, 45 (1956).
GORDEN, PH., ROBERTSON, G. L., SEEGMILLER, J. E.: Hyperuricemia, a concomitant of congenital vasopressin-resistant diabetes insipidus in the adult. Studies of uric acid metabolism and plasma vasopressin. New Engl. J. Med. **284**, 1057 (1971).
HOLLIDAY, M. A., BURSTIN, C., HURRAH, J.: Evidence that the antidiuretic substance in the plasma of children with nephrogenic diabetes insipidus is ADH. Pediatrics **32**, 384 (1963).
— — TAVOULARIS, L., POPPE, L.: An assay for ADH in which levels were demonstrated in jugular vein plasma of children with nephrogenic diabetes insipidus. Clin. Res. **10**, 228 (1962).
LINNEWEH, F., BUCHBORN, E., DELBRÜCK, B.: Familiärer renaler Diabetes insipidus. Klin. Wschr. **35**, 321 (1957).
— JARAUSCH, K. H.: Diabetes insipidus renalis. Klin. Wschr. **39**, 501 (1961).
MACDONALD, W. B.: Congenital pitressin resistant diabetes insipidus of renal origin. Pediatrics **15**, 298 (1955).
ORLOFF, J., BURG, M. B.: Vasopressin-resistant diabetes insipidus. In: STANBURY, WYNGAARDEN, FREDRICKSON. The metabolic basis of inherited disease. 3. ed. New York: McGraw Hill 1972.

Lithium-Induced Nephrogenic Diabetes Insipidus

Editorial: Lithium-induced diabetes insipidus. Brit. med. J. **1972II**, 726.
SINGER, I., ROTENBERG, D., PUSCHETT, J. B., FRANKO, E. A.: Lithium-induced nephrogenic diabetes insipidus: in vivo and in vitro studies. J. clin. Invest. **51**, 1081 (1972).

Transitory Diabetes Insipidus

HANKISS, J., KESZTHELYI, M., SIRO, B.: A new type of diabetes insipidus due to increased hormone inactivation: its incidence in clinical material. Amer. J. med. Sci. **242**, 603 (1961).
KEDES, H., BONESSI, L. J. V., DANOWSKI, T. S.: Intermittent supraoptic-hypophyseal diabetes insipidus with acquired pitressin resistance. Ann. intern. Med. **61**, 1128 (1964).
ROTH, J., GLICK, S. M., KLEIN, L. A., PETERSEN, M. J.: Specific antibody to vasopressine in man. J. clin. Endocr. **26**, 671 (1966).

Polydipsia— Adipsia, Central Hyperelectrolytemia, Diabetes Insipidus Occultus

AVIOLI, L. V., EARLY, L. E., KASHIMA, H. K.: Chronic and sustained hypernatremia, absence of thirst, diabetes insipidus and adrenocorticotrophin insufficiency resulting from wide spread destruction of the hypothalamus. Ann. intern. Med. **56**, 131 (1962).
BARLOW, E. D., WARDENER, H. E. DE: Compulsive water drinking. Quart. J. Med. **28**, 235 (1959).
COOKE, R. E., OTTENHEIMER, E. J.: Clinical and experimental interrelations of sodium and the central nervous system. Advanc. Pediat. **11**, 81 (1960).
COOPER, I. S., CERVIER, P. H.: Neurogenic hypernatremia and hyperchloremia. J. clin. Endocr. **12**, 821 (1952).
DECOURT, J., BERNARD-WEIL, E., MICHARD, J. P.: Sur la guérison possible par une méthode combinée de persuasion et de conditionnement de syndromes polyuro-polydipsiques présentant certains critères considerés comme caractéristiques des diabètes insipides vrais. Sem. Hôp. Paris **36**, 361 (1960).

FANCONI, G.: Zur Differentialdiagnose des Diabetes insipidus. Helv. paediat. Acta **11**, 506 (1956).

FILLASTRE, J. P., POCIDALO, J. J., SALET, J.: Syndrome polyuropolydipsique d'allure potomaniaque évoluant vers l'adipsie. Presse méd. **76**, 1103 (1968).

GILBOA, Y., VRIES, A. DE: Psychogenic polydipsia cured by water intoxication following vasopressin. Arch. intern. Med. **104**, 290 (1959).

HOGAN, P. A., WOOLSEY, R. M.: Polydipsia associated with occult hydrocephalus. New Engl. J. Med. **277**, 639 (1967).

KAPPELER, H. J., FROESCH, E. R., LABHART, A.: Fehleinstellung des Durstzentrums. Zur Differentialdiagnose zwischen organisch bedingter Polydipsie und echtem Diabetes insipidus. Helv. med. Acta **31**, 437 (1964).

KASTIN, A. J., LIPSETT, M. B., OMMAYA, A. K., MOSER, J. M.: Asymptomatic hypernatriemia. Amer. J. Med. **38**, 306 (1965).

LINQUETTE, M., LAINE, E., FOSSATI, P., LEFÈBVRE, J.: Pinéalome ectopique avec insuffisance hypophysaire antérieure, adipsie et hypernatriémie neurogène. Ann. Endocr. (Paris) **28**, 39 (1967).

MAHONEY, J. H., GOODMAN, A. D.: Hypernatremia due to hypodipsia and elevated treshold for vasopressin release. Effects of treatment with hydrochlorothiazide, chlorpropamide and tolbutamide. New Engl. J. Med. **279**, 1191 (1968).

PRADER, A., ISLER, W.: Chronische neurogene Hyperelectrolytämie infolge pränataler Hirnschädigung. Helv. paediat. Acta **9**, 264 (1954).

RICHNER, K.: Diabetes insipidus occultus. Schweiz. med. Wschr. **100**, 2100 (1970).

RODECK, H.: Diabetes insipidus und primäre Oligurie (Antidiabetes insipidus). Ergebn. inn. Med. Kinderheilk. **6**, 185 (1956).

SAITO, T., YOSHIDA, S., NAKAO, H., TAKANASHI, R.: Chronic hypernatremia associated with inflammation of the neurohypophysis. J. clin. Endocr. **31**, 391 (1970).

TRUNIGER, B., KÜNZLER, D.: Chronische Hyperosmolarität bei Hypothalamusläsion. Zur Pathogenese des Diabetes insipidus hypersalaemicus occultus. Klin. Wschr. **40**, 872 (1962).

WEBER, G.: Tumoren der Glandula pinealis und ektopische Pinealozytome. Schweiz. Arch. Neurol. Neurochir. Psychiat. **91**, 473 (1963).

WELT, L.: Hypo- and Hypernatremia. Ann. intern. Med. **56**, 161 (1962).

ZIERLER, K. L.: Hyperosmolarity in adults: A critical review. J. chron. Dis. **7**, 1 (1958).

Diagnosis

ALEXANDER, C. S., FILBIN, D. M.: Extraction and bioassay of antidiuretic hormone from human urine by a new method. J. Lab. clin. Med. **60**, 855 (1962).

CHAUDHURY, R. R., CHUTTANI, H. K., RAMALINGASWAMI, V.: The antidiuretic hormone and liver damage. Clin. Sci. **21**, 199 (1961).

CZACZKES, J. W., KLEEMAN, C. R., KOENIG, M.: Physiologic studies of antidiuretic hormone by its direct measurement in human plasma. J. clin. Invest. **43**, 1625 (1964).

DASHE, A. M., CRAMM, R. E., CRIST, CH. A., HABENER, J. F., SOLOMON, D. H.: A water deprivation test for the differential diagnosis of polyuria. J. Amer. med. Ass. **185**, 699 (1963).

DECOURT, J., BERNARD-WEIL, E., HUREZ, D., ROBEL, P.: Epreuves comparées d'ingestion d'eau avec ou sans sel, chez des sujets normaux, des sujets atteints de diabète insipide et des polydipsiques purs. Intérêt pratique du test. Ann. Endocr. (Paris) **21**, 470 (1960).

DIES, F., RANGEL, S., RIBERA, A.: Differential diagnosis between diabetes insipidus and compulsive polydipsia. Ann. intern. Med. **54**, 710 (1961).

DINGMAN, J. F., BENIRSCHKE, K., THORN, G. W.: Studies of neurohypophyseal function in man. Diabetes insipidus and psychogenic polydipsia. Amer. J. Med. **23**, 226 (1957).

DYKE, H. B., VAN, ADAMSON, K., JR., ENGEL, S. L.: Aspects of the biochemistry and physiology of neuro-hypophyseal hormones. Recent Progr. Hormone Res. **11**, 1 (1955).

GARROD, O.: Clinical tests of neurohypophysial antidiuretic function. In: HARRIS and DONOVAN, The pituitary, vol. III. London: Butterworths 1966.

HEINTZ, R., DREWS, E. F., DREWS, G., BRASS, H.: Untersuchungen über den Gehalt des menschlichen Plasmas an antidiuretischem Hormon mit einer verbesserten Methode zum Hormonnachweis. Klin. Wschr. **42**, 771 (1964).

IRMSCHER, K., BREITBACH, A.: Untersuchungen zur Spezifität des Adiuretin-Nachweises an der alkoholnarkotisierten, hydratisierten Ratte mit einer modifizierten Registriertechnik. Z. ges. exp. Med. **139**, 286 (1965).

JADRESIC, A., MAIRA, J.: A simple test for the diagnosis of diabetes insipidus. Lancet **1962I**, 402.

LAUSON, H. D.: Vasopressin and oxytocin in the plasma of man and other mammals. In: ANTONIADES, H., Hormones in human plasma. Boston: Little Brown 1960.

MOSES, A. M., STREETEN, D. H. P.: Differentiation of polyuric states by measurement of responses to changes in plasma osmolality induced by hypertonic saline infusions. Amer. J. Med. **42**, 368 (1967).

OYAMA, S. N., KAGAN, A., GLICK, S. M.: Radioimmunoassay of vasopressin: Application to unextracted human urine. J. clin. Endocr. **33**, 739 (1971).

PRICE, J. D. E., LAUENER, R. W.: Serum and urine osmolalities in the differential diagnosis of polyuric states. J. clin. Endocr. **26**, 143 (1966).

ROBERTSON, G. L.: Radioimmunoassay of plasma vasopressin in man. Clin. Res. **19**, 562 (1971).

— KLEIN, L. A., ROTH, J., GORDEN, PH.: Immunoassay of plasma vasopressin in man. Proc. nat. Acad. Sci. (Wash.) **66**, 1298 (1970).

ROBINSON, A. G., ZIMMERMAN, E. A., ENGLEMAN, E. G., FRANTZ, A. G.: Radioimmunoassay of bovine neurophysin: Specifity of neurophysin I and neurophysin II. Metabolism **20**, 1138 (1971).

ROMANI, J. D., ALBEAUX-FERNET, M.: Les conditions de l'osmolarité plasmatique dans les syndromes polyuropolydipsiques. Presse méd. **73**, 277 (1965).

ROTH, J., GLICK, S. M., KLEIN, L. A., PETERSEN, M. J.: Specific antibody to vasopressin in man. J. clin. Endocr. **26**, 671 (1966).

SAWYER, W. H.: Biological assay for neurohypophysial principles in tissues and in blood. In: HARRIS and DONOVAN, The pituitary gland. London: Butterworths 1966.

STUDER, H., PFENNINGER, A.: Etudes clinique des polyuries d'origine hypothalamo-hypophysaire. Helv. med. Acta **26**, 720 (1959).

STÜRMER, E.: Bioassay procedures for neurohypophysial hormones and similar polypeptids. In: Handbuch der experimentellen Pharmakologie, vol. XXIII, ed. by B. BERDE. Berlin-Heidelberg-New York: Springer 1968.

Therapy

ANDERSSON, K. E., ARNER, B.: Effects of DDAVP, a synthetic analogue of vasopressin, in patients with cranial diabetes insipidus. Acta med. scand. **192**, 21 (1972).

ANDREANI, D., CINOTTI, G. H., STIRATI, G.: Chlorpropamide in idiopathic diabetes insipidus. Metabolism **18**, 874 (1969).

ARDUINO, F. F., FERRAZ, P. J., RODRIGUES, J.: Antidiuretic action of chlorpropamide in idiopathic diabetes insipidus. J. clin. Endocr. **26**, 325 (1966).

BERNDT, W.O., MILLER, M., KETTYLE, W.M., VALTIN, H.: Potentiation of antidiuretic effect of vasopressin by chlorpropamide. Endocrinology **68**, 1028 (1970).

BRAUNHOFER, J., ZICHA, L.: Eröffnet Tegretal neue Therapiemöglichkeiten bei bestimmten neurologischen und endokrinen Krankheitsbildern? Med. Welt **17**, 1875 (1966).

BÜTIKOFER, E., DE WECK, A.L., SCHERRER, M.: "Pituitary snuff taker's lung". Schweiz. med. Wschr. **100**, 97 (1970).

CRAWFORD, J.D., KENNEDY, G.C., HILL, L.E.: Clinical results of treatment of diabetes insipidus with drugs of the chlorothiazid series. New Engl. J. Med. **262**, 737 (1960).

CUTTING, H.O.: Inappropriate secretion of antidiuretic hormone secondary to vincriscine therapy. Amer. J. Med. **51**, 269 (1971).

DECOURT, J.: Avantage du clofibrate dans un cas de diabète insipide. Ann. Endocr. (Paris) **32**, 284 (1971).

DINGMAN, J.F., HANGER-KLEVENE, J.H.: Treatment of Diabetes insipidus: synthetic lysine-vasopressin nasal solution. J. clin. Endocr. **24**, 550 (1964).

EARLEY, L.E.: Chlorpropamide antidiuresis. Editorial, New Engl. J. Med. **284**, 103 (1971).

— KAHN, M., ORLOFF, J.: The effects of infusions of chlorothiazide on urinary dilution and concentration in the dog. J. clin. Invest. **40**, 857 (1961).

— ORLOFF, J.: The mechanism of antidiuresis associated with the administration of hydrochlorothiazide to patients with vasopressin-resistant diabetes insipidus. J. clin. Invest. **41**, 1988 (1962).

FRAHM, H., SMEJKAL, E., KRATZENSTEIN, R.: Antidiuretic effect of an anticonvulsant drug (5-Carbamyl-5H-dibenzo(B, F)azepin = Tegretal) associated with measurable increase of ADH activity in serum of patients suffering from diabetes insipidus and of patients with polyuria and polydipsia following hypophysectomy. Acta endocr. (Kbh.), Suppl. **138**, 240 (1969).

FRØYSHOV, I., HAUGEN, H.N.: Chlorpropamide in diabetes insipidus. Acta med. scand. **183**, 397 (1968).

GOODMAN, A.D., CARTER, R.D.: A study on the mechanism of the antidiuretic action of chlorothiazide in diabetes insipidus. Metabolism **11**, 1033 (1962).

HANEFELD, F., LEVSEN, I., STEFAN, H.: Untersuchungen zur Wirkung von Carbamazepin auf die neurosekretorischen Kerne der Ratte. Z. ges. exp. Med. **153**, 95 (1970).

HARRINGTON, A.R., VALENTIN, H.: Impaired urinary concentration after vasopressin and its gradual correction in hypothalamic diabetes insipidus. J.. clin. Invest. **47**, 502 (1968).

HAVARD, C.W.H., WOOD, PH. H.N.: Antidiuretic properties of hydrochlorothiazide in diabetes insipidus. Brit. med. J. **1960 I**, 1306.

HOCKEN, A.G., LONGSON, D.: Reduction of free water clearance by chlorpropamide. Brit. med. J. **1968 I**, 355.

INGELFINGER, J.R., HAYS, R.M.: Evidence that chlorpropamide and vasopressin share a common site of action. J. clin. Endocr. **29**, 738 (1969).

IRMSCHER, K., WIEGELMANN, W., BETHGE, A., KAMPFFMEIER, J., SOLBACH, H.G., ZIMMERMANN, H.: Therapeutic response of central diabetes insipidus on nasal application of a new synthetic analogue of vasopressin (1-deamino-8-D-arginine-vasopressin, DDAVP). Acta endocr. (Kbh.), Suppl. **159**, 52 (1972).

MOSES, A.M., HOWANITZ, J., VAN GEMERT, M., MILLER, M.: Clofibrate—induced antidiuresis. J. clin. Invest. **52**, 535 (1973).

PERLEMUTTER, L., HAZARD, J.: Diabète insipide de l'adulte. La nouvelle Presse méd. **79**, 2445 (1971).

STOLL, E., LARGIER, H., TÖNZ, O.: Behandlung eines kindlichen Diabetes insipidus centralis mit Chlorpropamid (Diabinese) und Carbamazepin (Tegretol). Schweiz. med. Wschr. **102**, 10 (1972).

THOMAS, W.C., JR.: Diabetes insipidus. J. clin. Endocr. **17**, 565 (1957).

UHLICH, E., EIGLER, J., TRYZNA, G., FINKE, K., WINKELMANN, W., BUCHBORN, E.: Zum Wirkungsmechanismus von Chlorpropamid bei Diabetes insipidus. Klin. Wschr. **49**, 314 (1971).

VAVRA, I., MACHOVA, A., HOLECEK, V., CORT, J.H., ZAORAL, M., SORM, F.: Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. Lancet **1968 I**, 948.

WEISSMAN, P.N., SHENKMAN, L., GREGERMAN, R.I.: Chlorpropamide hyponatremia. Drug-induced inappropriate antidiuretic-hormone activity. New Engl. J. Med. **284**, 65 (1971).

WILSON, M.L., MCGINTY, D.A.: The duration of antidiuretic action of pitression solution and of pitressin tannate in aqueous and oil suspensions. J. clin. Endocr. **11**, 963 (1951).

Inappropriate Secretion of Vasopressin (SCHWARTZ-BARTTER)

AMATRUDA, TH. T., JR., MULROW, P.J., GALLAGHER, J.C., SAWYER, W.H.: Carcinoma of the lung with inappropriate antidiuresis. Demonstration of antidiuretic-hormone-like activity in tumor extract. New Engl. J. Med. **269**, 544 (1963).

AVIRAM, A., PFAU, A., CZACZKES, J.W., ULLMANN, T.D.: Hyperosmolality with hyponatremia, caused by inappropriate administration of mannitol. Amer. J. Med. **42**, 648 (1967).

BARRACLOUGH, M.A., JONES, J.J., LEE, J.: Production of vasopressin by anaplastic oat cell carcinoma of the bronchus. Clin. Sci. **31**, 135 (1966).

BARTTER, F.C., SCHWARTZ, W.B.: The syndrome of inappropriate secretion of antidiuretic hormone. Amer. J. Med. **42**, 790 (1967).

BOWER, B.F., MASON, D.M.: Measurement of antidiuretic activity in plasma and tumor of the lung with inappropriate antidiuresis. Clin. Res. **12**, 121 (1964).

BRICKER, N.S.: The control of sodium excretion with normal and reduced nephron populations. The pre-eminence of third factor. Amer. J. Med. **43**, 313 (1967).

CARTER, N.W., RECTOR, F.C., JR., SELDIN, D.W.: Hyponatriema in cerebral disease resulting from inappropriate secretion of antidiuretic hormon. New Engl. J. Med. **264**, 67 (1961).

CUTLER, R.E., KLEEMAN, C.R., MAXWELL, M.H., DOWLING, J.T.: Physiologic studies in nephrogenic diabetes insipidus. J. clin. Endocr. **22**, 827 (1962).

FICHMAN, M.P., BETHUNE, J.E.: The role of the adrenocorticoids in the inappropriate antidiuretic hormone syndrome. Ann. intern. Med. **68**, 806 (1968).

GOLDBERG, M.: Hyponatremia and the inappropriate secretion of antidiuretic hormone. Amer. J. Med. **35**, 293 (1963).

IVY, H.K.: Renal sodium loss and bronchogenic carcinoma. Arch. intern. Med. **108**, 115 (1961).

JAENIKE, J.R., WATERHOUSE, C.: The renal response to sustained administration of vasopressin and water in man. J. clin. Endocr. **21**, 231 (1961).

KAYE, M.: An investigation into the cause of hyponatremia in the syndrome of inappropriate secretion of antidiuretic hormone. Amer. J. Med. **41**, 910 (1966).

MARKS, L.J., BERDE, B., KLEIN, L.A., ROTH, J., GOONAN, S.R., BLUMEN, D., NABSETH, D.C.: Inappropriate vasopressin secretion and carcinoma of the pancreas. Amer. J. Med. **45**, 967 (1968).

MILLER, M., MOSES, A.M.: Urinary antidiuretic hormone in polyuric disorders and in inappropriate ADH syndrome. Ann. intern. Med. **77**, 715 (1972).

PFENNINGER, A., MICHELI, H., MÜLLER, A.F.: Carcinome bronchique et secrétion inappropriée d'hormone antidiurétique (syndrome de SCHWARTZ-BATTER). Helv. med. Acta **30**, 640 (1963).

Ross, E. J.: Hyponatraemic syndromes associated with carcinoma of the bronchus. Quart. J. Med., N.S. **32**, 297 (1963).

Ruch, W.: Estimation of antidiuretic hormone in the urine of healthy subjects and patients with inappropriate secretion of vasopressin (Schwartz-Bartter Syndrome). Acta endocr. (Kbh.) **54**, 113 (1967).

Schwartz, W. B., Bennett, W., Curelop, S., Bartter, F. C.: A syndrome of renal sodium loss and hyponatremia probably resulting from inappropriate secretion of antidiuretic hormone. Amer. J. Med. **23**, 529 (1957).

Sousa, R. C. de, Jenny, M.: Hyponatriémie par dilution dans un cas de carcinome pancréatique. Schweiz. med. Wschr. **94**, 930 (1964).

Added in Proofs:

Edwards, C. R. W., Kitau, M. J., Chard, T., Besser, G. M.: Vasopressin analogue DDAVP in diabetes insipidus: Clinical and Laboratory studies. Brit. med. J. **3**, 375 (1973).

Thorn, N. Y., Transbøl, I.: Hyponatremia and bronchogenic carcinoma associated with renal excretion of large amounts of antidiuretic material. Amer. J. Med. **35**, 257 (1963).

Utiger, R. D.: Inappropriate antidiuresis and carcinoma of the lung: detection of arginine vasopressin in tumor extracts by immunosassay. J. clin. Endocr. **26**, 970 (1966).

Vorherr, H., Massry, S. G., Utiger, R. D., Kleeman, C. R.: Antidiuretic principle in malignant tumor extracts from patients with inappropriate ADH-syndrome. J. clin. Endocr. **28**, 162 (1968).

Waldvogel, F., Sousa, R. C. de, Mach, R. S.: Intoxication à l'eau due à une sécrétion inadéquate d'hormone antidiurétique (syndrome de Schwartz-Bartter) d'origine idiopathique. Schweiz. med. Wschr. **97**, 929 (1967).

IV. The Pineal Body and the Circumventricular Organs

A. LABHART

A. Pineal Body

Throughout the history of medicine, opinions on the importance and functions of the pineal body have varied more than those related to any other organ of the body. DESCARTES saw it as the seat of the functions of the soul, imagining that light impulses could be perceived by the pineal body and then transmitted as humoral signals to the musculature. From this position of eminence, theories concerning its function have relegated it to a position where it presently serves primarily as a calcified point of orientation in the middle of the skull for the neuroradiologists. At present it is receiving renewed importance as a potential fourth neuroendocrine organ, the biological clock of the organism and the rhythm initiator.

The phylogenetic history of this organ is also interesting. A million years ago, in the brontosaurians, this organ represented a third eye. It is still active as a light receptor in certain amphibians in that it contains a light-sensitive apparatus similar to the retinal cones which emit nerve impulses to light stimuli of certain wavelengths. The pineal body has become the neuroendocrine organ in mammals which need nerve impulses for the liberation of a specific hormone, melatonin. The indole alkylamine, melatonin (see Fig. 1), produced in small amounts, is the most potent pigment regulator in amphibians, causing aggregation of melanin granules in the melanophores and fading of skin pigmentation. In the mammal, melatonin has no influence on pigment regulation.

However, in the mammal the pineal body has not forgotten its past as a third eye, and although it no longer possesses any light-sensitive elements, its functions are still dependent upon light. Light impulses from the retina of the eye reach the pineal body through the sympathicus via the superior cervical ganglion where noradrenaline and serotonin are released. This is the only site where serotonin is released as a neurotransmitter. Noradrenaline and serotonin influence the synthesis of the enzyme 5-hydroxyindole O-methyl-transferase (HIOMT) which liberates melatonin from N-acetyl-serotonin. It is presently uncertain to what extent the limbic system influences the pineal body and through this means, further transmits olfactory stimuli (RELKIN, 1966).

The physiological function of melatonin in the mammal is still not clear. It is certain that it has no function in pigment regulation. It can, however, be shown to inhibit gonadal function, exerting a reducing action on the ovary, and delaying or inhibiting estrus in the rodent. Pinealectomy, darkness or division of the conveying sympathetic nervous pathway leads to loss of HIOMT, and thus also of melatonin, and to accelerated estrus. Melatonin administration reverses this acceleration. The effect of light on testicular size is also transmitted by the pineal body (REITER, 1968). The end organ of melatonin is uncertain. It acts on the brain, perhaps especially on the eminentia mediana (MARTINI, 1968), by influencing synthesis or release of one or more neurotransmitters (WURTMAN, 1971). Labeled melatonin accumulates mainly in the ovary, but also in the vagina, the brain and the pituitary gland.

In addition to the seasonal regulation of the estrus, the pineal body also appears to play an important part in the daily rhythm of the endocrine system, dependent upon light. Through the perception of light via the retina and the sympathetic system, the concentration of HIOMT and therewith the content of melatonin in the pineal body rises to its maximum at midnight. The concentration is lowest at midday. The rhythm disappears completely with continual illumination or continual darkness. A contrasting rhythm appears to be present in serotonin, the precursor, and in a certain sense possibly an antagonist. The content of serotonin in the pineal body is highest at midday and lowest at midnight. In this case, however, the rhythm is determined in the central nervous system: the light works only as a synchronizer, since the rhythm for serotonin does not disappear with light but only in the dark.

The pineal body begins to deposit calcium after puberty. This does not, however, appear

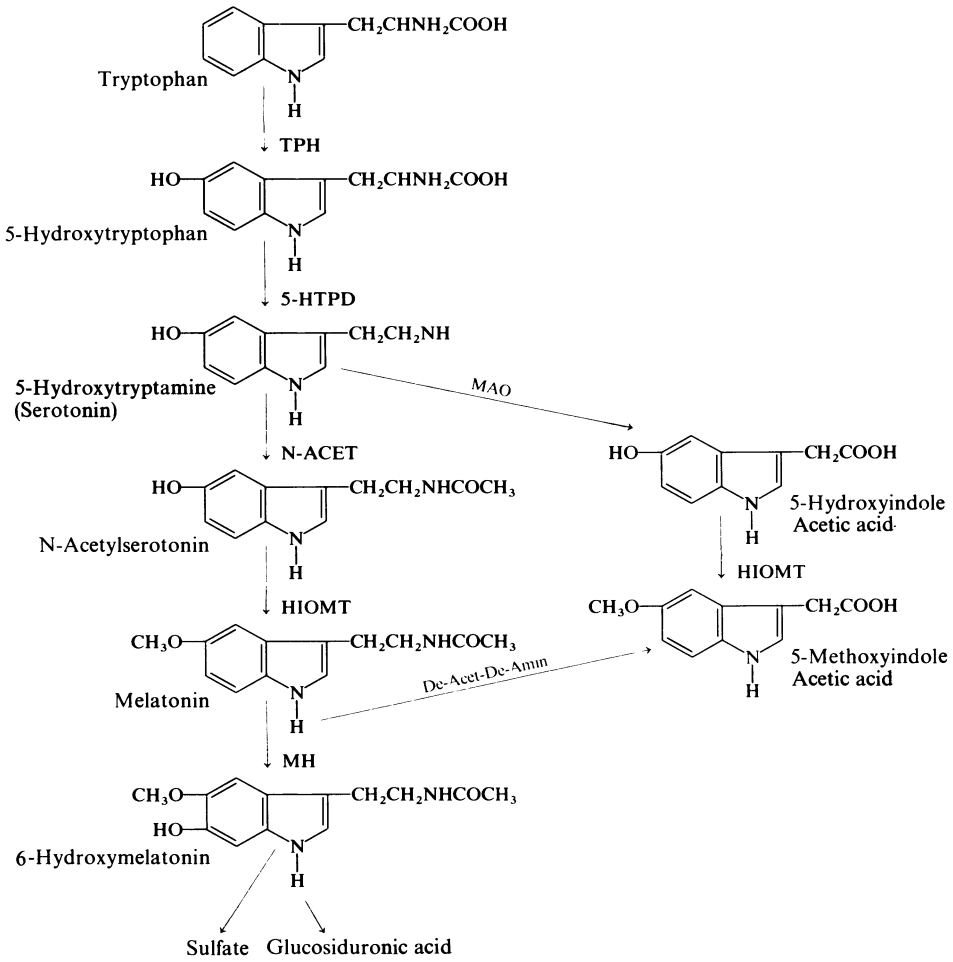


Fig. 1. Biosynthesis and metabolism of Melatonin. *TPH* tryptophan hydroxylase; 5-*HTPD* 5-hydroxytryptophan decarboxylase; *MAO* monoamine oxidase; *N-ACET.* N-acetylating enzyme; *HIOMT* hydroxyindole-O-methyltransferase- *DE-ACET.-DE-AMIN.* deacetylating and deaminating enzyme; and *MH* microsomal hydroxylase. (After RELKIN, 1966)

to influence its activity, since the activity of the decisive enzyme HIOMT remains intact from early youth to advanced age. Although the physiological significance in man is unknown, the knowledge obtained in the past few years has thrown new light on the known pathophysiological phenomena of puberty. Precocious puberty usually develops with tumors of the pineal region, although only if the tumors derive from the interstitium or a teratoma is involved. Tumors arising from the parenchyma of the pineal body lead to delayed puberty, and in at least two cases increased amounts of HIOMT have been demonstrated in these tumors. The pineal body may therefore be responsible for preventing the premature development of puberty and sexual function. It is possible that the accelerated development (Chap. XIX) observed nowadays is due to the increased light stimuli on the pineal body. There may be other antigonadotropins besides melatonin (BENSON, 1971; CHEESMAN, 1970). In addition to its influence on the pituitary-gonadal axis, the pineal body may have a role in the circadian rhythm of adrenocortical secretions. Melatonin, 5-methoxytryptophol and 5-hydroxytryptophol injected into the lateral cerebral ventricles decrease plasma corticosterone in the rat. This effect is possibly mediated via the adrenergic system (MOTTA, 1971).

As with serotonin, there are at present no known actions of melatonin on the psyche, nor is there any evidence that either of these substances is involved in the development of psychoses.

For a review of the physiology of the mammalian pineal body see REITER (1969).

Pinealomas without endocrine activity usually lead to two or more of the following symptoms: diabetes insipidus, hypopituitarism, calcifica-

tions in the region of the pineal body, deformation of the third ventricle with or without hydrocephalus. The incidence of pinealomas is highest in men before the age of twenty (PUSCHETT, 1968).

B. Subcommissural Organ

The subcommissural organ is a group of cells containing secretory granules which lie in the vicinity of the pineal body, under the pineal recess, at the caudal end of the roof of the third ventricle. In rats, it appears that these cells release a relatively insoluble substance into the cerebrospinal fluid and possibly also into the capillaries. Extracts from this region are believed to inhibit thirst and water intake. Patients with tumors in this pretectal region are supposed not to react to salt withdrawal with the normal increase of the aldosterone secretion. At present, research into the role of this organ in salt and water regulation is not sufficiently advanced for definite conclusions.

The subcommissural organ may belong to a system of "circumventricular organs" which includes the pineal body, the subfornical organ, the supraoptic crest, and certain special ependymal structures of the third ventricle. These various organs appear to possess certain morphological and functional similarities.

References

Epiphysis

AXELROD, J., WURTMAN, R.: The formation, metabolism and some actions of melatonin, a pineal gland substance. In: LEVINE, R., Endocrines and the central nervous system. Baltimore: Williams & Wilkins 1966.

— — Photic and neural control of indoleamine metabolism in the rat pineal gland. In: Advances in pharmacology, vol. 6, part A (E. COSTA and M. SANDLER, eds.), p. 141—151. New York and London: Academic Press 1968.

BAJUSZ, E.: Pineal gland and its control, p. 462, in: Clinical neuroendocrinology. Basel-New York: Karger 1967.

BENSON, B., MATTHEWS, M.J.: Studies on a non melatonin pineal antigonadotropin. 53rd Meet. Endocr. Soc. San Francisco, June 24–26 (1971).

CHEESMAN, D.W., FARISS, B.L.: Isolation and characterization of a gonadotropin-inhibiting substance from the bovine pineal gland. Proc. Soc. exp. Biol. (N.Y.) **133**, 1254 (1970).

KAPPERS, A.J., SCHADÉ, J.P.: Structure and function of the epiphysis cerebri. Progr. Brain Res. **10**, 87 (1965).

KAPPERS, J.A.: The morphological and functional evolution of the pineal organ during its phylogenetic development. In: Progress in endocrinology, ed. by GUAL. Amsterdam: Excerpta Medica Foundation 1969.

MOTTA, M., SCHIAFFINI, O., PIVA, F., MARTINI, L.: Pineal principles and the control of adrenocorticotropin secretion. In: The pineal gland (WOLSTENHOLME, G.E.W., KNIGHT, J., eds.), Edinburgh and London: Churchill, Livingstone 1971.

KITAY, J.I., ATSCHULE, M.D.: The pineal gland. Cambridge: Harvard University Press 1954.

PALKOVITS, M.: Participation of the epithalamo-epiphyseal system in the regulation of water and electrolytes metabolism. In: Structure and function of the epiphysis cerebri, ed. by J.A. KAPPERS and J.P. SCHADÉ. Amsterdam: Elsevier 1965.

PUSCHETT, J.B., GOLDBERG, M.: Endocrinopathy associated with pineal tumor. Ann. intern. Med. **69**, 203 (1968).

QUAY, W.B.: Circadian rhythm in rat pineal serotonin and its modifications by estrous cycle and photoperiod. Gen. comp. Endocr. **3**, 473 (1963).

— 24 hour rhythms in pineal 5-hydroxytryptamine and hydroxyindole-O-methyl transferase activity in the macaque. Proc. Soc. exp. Biol. (N.Y.) **121**, 946 (1966).

REICHLIN, S.: Neuroendocrinology. In: WILLIAMS, Textbook of endocrinology, 4th ed. Philadelphia: Saunders 1968.

REITER, R.J., FRASCHINI, F.: Endocrine aspects of the mammalian pineal gland: A review. Neuroendocrinology **5**, 219 (1969).

— SORRENTINO, S.D., HOFFMANN, J.C., RUBIN, P.H.: Pineal, neural and photic control of reproductive organ size in early androgen-treated male rats. Neuroendocrinology **3**, 246 (1968).

RELKIN, R.: The pineal gland. New Engl. J. Med. **274**, 1944 (1966).

SOFFER, L.J., FOGEL, M., RUDAVSKY, A.Z.: The presence of a gonadotrophin inhibiting substance in pineal gland extract. Acta endocr. (Kbh.) **48**, 561 (1965).

WEBER, G.: Tumoren der glandula pinealis und ektopische Pinealocytome. Schweiz. Arch. Neurol. Neurochir. Psychiat. **91**, 473 (1963).

WOLSTENHOLME, G.E.W., KNIGHT, J. (editors): The pineal gland. Edinburgh and London: Churchill, Livingstone, 1971.

WURTMANN, R.J., ANTON-TAY, F.: The mammalian pineal as a neuroendocrine transducer. Recent Progr. Hormone Res. **25**, 493 (1969).

— AXELROD, D.: The pineal gland. Sci. Amer. **213**, 1, 50 (1965).

— — The formation, metabolism and physiologic effects of melatonin. In: Advances in pharmacology, vol. 6, part A (E. COSTA and M. SANDLER eds.), pp. 141. New York and London: Academic Press 1968.

— AXELROD, J., KELLY, D.E.: The pineal. New York: Academic Press 1968.

Subcommissural Organ

GILBERT, G.J.: The subcommissural organs and water electrolyte metabolism. In: WAYNE, M.J., 1st internat. symposium on thirst in the regulation of body water, p. 457. New York: Macmillan 1964.

PALKOVITS, M., IMKE, G., LUKACS, C.: Der Subcommissuralkomplex (subcommissural organ) mit seinen Komponenten und Nachbargebilden des Menschen während der postnatalen Lebensperioden. Z. mikr.-anat. Forsch. **69**, 88 (1962).

REICHLIN, S.: Neuroendocrinology. In: WILLIAMS, R., Textbook of endocrinology, 4th ed. Philadelphia: Saunders 1968.

UPTON, P.D., DUNIHUE, F.W., CHAMBERS, W.F.: Subcommissural organ and water metabolism. Amer. J. Physiol. **201**, 711 (1961).

WISLOCKI, G.B., LEDUC, E.H.: The cytology of the subcommissural organ, REISNER's fiber, periventricular glial cells and posterior collicular recess of the rat's brain. J. comp. Neurol. **101**, 283 (1954).

V. The Adenohypophysis

A. LABHART

With Contributions by

CHR. HEDINGER, G. KISTLER, A. PRADER, G. TÖNDURY, and M. ZACHMANN

A. Historical Dates

1543 VESAL described the hypophysis as "glandula pituitaria cerebri excipiens".
1864 VERGA and
1884 FRITZSCHE and KLEBS described the clinical and pathological features of a case of acromegaly.
1886 PIERRE MARIE named the illness "acromegaly".
1900 BENDA associated acromegaly with an eosinophil hypophyseal adenoma.
1909 ASCHNER showed that hypophysectomy in a growing animal resulted in dwarfism.
1921 EVANS and LONG, using pituitary extract, produced gigantism in rats.
1926 SMITH demonstrated pituitary control over the gonads.
1928 ASCHHEIM and ZONDEK described the gonadotropic hormones.
1928 STRICKER and GRUETER discovered prolactin.
1935 RIDDLE characterized prolactin.
1944 LI and EVANS succeeded in isolating crystallized growth hormone.
1955 KNOBIL and GREEP extracted growth hormones from monkeys which was active in man and showed the species-specificity of growth hormone.
1957 RABEN described a method for extracting human growth hormone from the hypophyses of cadavers.
1961 UTTIGER, PARKER and DAUGHADAY,
1962 HUNTER and GREENWOOD,
1963 GLICK, ROTH, YALOW and BERSON described a radioimmunological method for measuring human growth hormone.
1966–1971 LI described the complete amino-acid structure of human growth hormone and succeeded in synthesizing it.

B. Embryology and Anatomy

G. TÖNDURY and G. KISTLER

1. Embryology

In the human embryo of 6–8 somites, the primitive gut is closed at the cranial end by the pharyngeal membrane which forms the floor of an external depression, the stomadeum. At this site, ectoderm and entoderm come into direct contact. When the embryo is about 2.5 mm long (18–20 somites), the pharyngeal membrane ruptures and the stomadeum develops into the ectodermal portions of the mouth. At this stage, the stomadeal epithelium situated dorsally and just in front of the remnants of the pharyngeal membrane evaginates to form the RATHKE'S pouch, i.e., the anlage of the adenohypophysis. This outpocketing enlarges quickly towards the floor of the diencephalon. Its originally wide mouth soon becomes compressed by a proliferation of the surrounding mesenchyme. Thus, the anlage of the adenohypophysis becomes an epithelial vesicle which remains temporarily connected to the roof of the primitive oral cavity by a slender epithelial stalk.

When the embryo has reached a length of some 7–8 mm, an epithelial stalk grows downward from the anlage of the neurohypophysis in the diencephalon and contacts the upper and posterior portions of the adenohypophyseal vesicle. This leads to a marked proliferation of the front wall of the vesicle, which thickens to become the main cellular mass of the *anterior lobe*. From the rostral portions of this lobe, bilateral epithelial buds proliferate and grow upward to the floor of the third ventricle. The two buds fuse together to form the *pars infundibularis* (pars tuberalis) of the adenohypophysis. The buds often also encircle the root of the infundibulum. In contrast, the posterior wall of the adenohypophyseal vesicle remains thin. It differentiates into the *pars intermedia* of the glandular lobe. The original cavity of the vesicle becomes the *residual lumen* of the adenohypophysis. This cavity usually

disappears completely but may persist as multiple cysts filled with colloidal material throughout life. The epithelial stalk connecting the epithelium of the oral cavity with the adenohypophyseal vesicle in the early stages usually disintegrates. Sometimes, however, islets of these epithelial cells persist and form *accessory adenohypophyseal glands* which are usually situated within either the sella turcica or the sphenoid bone itself but may also be found in the pharyngeal roof. When present, the so-called *pharyngeal roof hypophysis* is usually the best developed of these accessory glands. It lies within the pharyngeal epithelium, exactly on the mid-line of the cavity, and is surrounded by small nerve fibers and blood vessels.

2. Gross Anatomy

The hypophysis of the human adult weighs about 600–700 mg and measures approximately 0.5 × 1 × 1 cm. Macroscopically, the organ can be divided not only into a neural and glandular portion, but also into a proximal and a distal subdivision separated from each other by the diaphragma sellae. The main portion is situated within the sella turcica of the sphenoid bone, where it is surrounded by a rather thick connective tissue capsule. Thus, the intrasellar portions of the hypophysis (anterior, posterior and intermediate lobe) are situated within the dura.

The diaphragma sellae separates the cavity of the sella turcica from the middle cranial fossa. This dural fold forms an opening, the foramen hypophyseale, which lies closely around the hypophyseal stalk. Above the diaphragm, the stalk passes through the peri-infundibular cistern, where it is surrounded by cerebrospinal fluid.

The hypophysis consists of two developmentally, morphologically, and functionally quite distinct parts. The neurohypophysis has already been described in Chap. III, p. 39. The *adenohypophysis* (lobus glandularis, anterior lobe) is derived from an outpocketing of the ectodermal epithelium of the primitive oral cavity (see p. 77). It shows all the characteristics of an endocrine gland. Three major subdivisions may be distinguished:

1. The *pars infundibularis* (pars proximalis or pars tuberalis of the adenohypophysis) is a subdivision of the anterior lobe. It extends from the diaphragma sellae up to the floor of the diencephalon, closely following the course of the infundibulum (pars proximalis of the neurohypophysis), from which it is incompletely separated by a thin sheet of connective tissue continuous with the organ capsule.

2. The *pars intermedia* (zona intermedia or intermediate lobe) is phylo- and ontogenetically a portion of the glandular lobe (see p. 80). It is situated in the sella turcica and is sandwiched between the secretory mass of the anterior lobe and the infundibular process (neural lobe). The intermediate zone forms the so-called distal adeno-neurohypophyseal contact which will be discussed below (see p. 82).

3. The *anterior lobe* (pars distalis of the adenohypophysis) constitutes the bulk of the glandular parenchyma. It is situated completely within the sella turcica.

3. Histology

The anterior and posterior lobes of the hypophysis are surrounded by a rather thick connective tissue capsule, the innermost layer of which is closely applied to the organ parenchyma. The next layer is rich in blood vessels and is therefore called the stratum vasculare. The outermost layer, the stratum periostale, belongs to the periosteum of the sella turcica. Even at low magnification, the deeply stained adenohypophysis stands out sharply against the less stained and more homogeneous posterior lobe. In the pars intermedia, follicles of various sizes containing colloidal material are usually observed. They are known as the Rathke's cysts.

The three subdivisions of the adenohypophysis (pars distalis, pars intermedia, and pars infundibularis) consist of irregular cords and clumps of epithelial cells which are in intimate contact with the dense network of sinusoidal capillaries. Small bundles of collagenous fibers accompany the arteries and portal venules. They are continuous with a fine network of reticular fibers which surround both the epithelial cords and the capillaries. The endothelial cells of the sinusoids are fenestrated. At these sites, the circulating blood is separated from the perivascular space and from the glandular tissue only by the extremely thin diaphragms of the fenestrae and the basal laminae of both the endothelium and the epithelial cords.

Pars Distalis. In contrast to the rather uniform cell populations of both the pars intermedia and the pars infundibularis, the various cell types of the pars distalis differ markedly in size, shape, and affinity for stains. In routine histological sections, chromophilic and chromophobic cells can easily be distinguished. According to the tinctorial reactions of their specific granules in sections stained with special dyes (e.g. alum-hematoxylin and eosin), the chromophilic cells can be further subdivided into

acidophils and basophils. As this affinity for the various stains reflects a characteristic property only of the granules and not of the cytoplasm itself, there is still some considerable controversy about the nomenclature. Identification of the cell types is perhaps best done by the PAS reaction, which is specific for the granules of the basophils and therefore allows these cells to the distinguished from the acidophils and the chromophobes. More recently, electron microscopy has permitted more accurate differentiation of the specific granules of the various cell types and their subclasses.

The *acidophils* (alpha cells) make up approximately 37–44% of the total cell population of the pars distalis (BARGMANN, 1964). They measure about 12–20 μm in diameter, are mainly rounded and contain a large number of granules, the size of which permits them to be resolved with the light microscope. Ultrastructurally, these cells display a well-developed, rough endoplasmic reticulum, a large Golgi complex and rather small, rod-shaped mitochondria. In various mammalian species, two classes of acidophils can be distinguished according to their staining reactions with azocarmine and orange-G. In the rat, the specific granules of these two cell classes also differ markedly in diameter, the one measuring approximately 3000–3500 Å and the other 6000–9000 Å. The number of cells with the smaller but more numerous granules remains fairly constant throughout life. It is believed that these elements produce growth hormone (STH). In contrast, the number of cells containing the larger but less numerous granules is obviously increased during pregnancy and lactation. It is therefore assumed that these cells synthesize prolactin (LTH, luteotropic hormone). The production of these protein hormones by the acidophilic cells, besides being confirmed by immunohistochemical methods, is also supported by pathological findings in humans. Thus, in children an acidophilic adenoma of the anterior lobe results in gigantism, whereas in the adult acromegaly results. Experimentally, removal of a litter from lactating rats leads to marked regressive changes in the prolactin-producing cells within 48–96 hours. Newly produced hormone granules no longer release their content into the perivascular space (and further into the capillaries), but enter the lysosomal cycle and are digested within the cell (SMITH and FARQUHAR, 1966).

The *basophils* (beta cells) of the anterior lobe vary considerably in size (15–25 μ) and

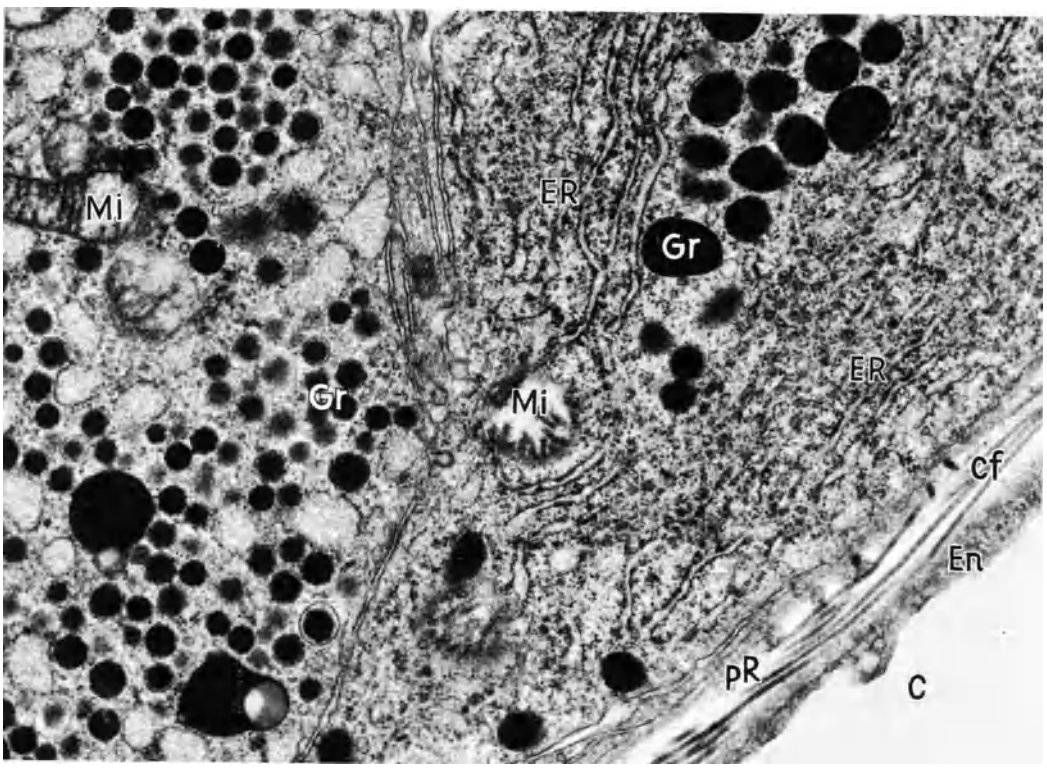


Fig. 1. Incretory cells of the anterior lobe of the rat. Note the difference in size of the hormone granules. *ER* rough endoplasmic reticulum; *C* capillary lumen; *En* endothelium; *pR* perivascular space; *cf* collagenous fibrils; *Gr* hormone granules; *Mi* mitochondria. × 20000

shape (round, oval, polygonal). Their diffusely distributed specific granules measure approximately 1000–2000 Å in diameter and are thus distinctly smaller than those of the acidophilic cells. Light microscopy barely reveals them. Since the hormones produced by the basophils are glycoproteins, the granules are best visualized by the periodic-acid-Schiff (PAS) reaction. They are, however, also stained by hematoxylin, aniline blue or resorcin-fuchsin. In most mammalian species, four types of basophils seem to be distinguishable, according to the size and the affinity of the granules for aldehyde-fuchsin. The so-called beta-basophil cell, which is presumed to produce thyrotropic hormone (TSH), stains with this dye. In the rat, its granules have a diameter of 1000–1500 Å and are situated mainly at the cell periphery. The somewhat larger delta-basophils contain an abundant endoplasmic reticulum and a well-developed Golgi apparatus. Their specific granules measure about 2000 Å in diameter and fail to stain with aldehyde-fuchsin. The delta-basophilic cells are the source of the gonadotropins, i.e., the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH or ICSH). In the rat, it was possible to distinguish the cells producing the two hormones morphologically (KUROSUMI and OOTA, 1968; ROOS, 1968). It has long been known that destruction of a peripheral endocrine organ is usually followed by hypertrophy of the corresponding cells in the anterior lobe. Thus, removal of the thyroid gland leads to enlargement and apparently also to an increase in number of the betabasophilis (= thyroidectomy cells). Similarly, castration is followed by an increase in size and by vacuolization of the gonadotropin-synthesizing delta-basophils (= castration cells).

There is some evidence that the basophils are also the source of the adrenocorticotropic hormone (ACTH, corticotropin). Whether its synthesis takes place within a specific cell type or occurs in the basophils which produce gonadotropins is still an open question. Interestingly enough, in the melanocyte-stimulating hormone (MSH) produced by the basophilic cells of the pars intermedia, the amino-acid sequence is similar or even identical to that of ACTH over large parts of the molecule. Release of these two hormones often seems to occur simultaneously and ACTH is known also to have a certain melanocyte-stimulating effect. Thus the increased skin pigmentation observed in adrenal insufficiency (Addison's disease) and pregnancy is thought to be the result of an overproduction of one or both of the hormones ACTH and MSH.

The chromophobes form the third large cell group of the hypophyseal anterior lobe. They are usually smaller than the acidophils and basophils and are almost devoid of specific granules. The function of these cells has been a matter of dispute for a long time. Because mitoses in the parenchyma of the adenohypophysis are very rare, the chromophobes used to be considered as pluripotent stem cells capable of differentiating into each of the various acidophils and basophils. Ultrastructural studies have, however, shown that the chromophobes do not constitute a uniform cell population. According to their nuclear and cytoplasmic characteristics (chromatin structure, size and number of mitochondria, form of the Golgi complex, etc.), a large proportion of these cells has to be grouped with the acidophils or basophils in spite of the absence of granules. It would therefore appear that most of the chromophobes are stem cells already determined in their differentiation capability. Others, however, seem to be mature chromophils degranulated to such an extent that they fail to stain with specific dyes. It is still not known whether such a degranulation represents a reversible functional state or precedes cell death.

Pars Intermedia. In the human fetus, the borderline zone between the anterior and the posterior lobe consists of a thin sheet of connective tissue and a stratified epithelium of small basophilic cells. These cells are derived from the posterior wall of the adenohypophyseal vesicle (see p. 77) and are, in turn, separated from the main incretory mass of the anterior lobe by a distinct cleft (the remnant of the lumen of the Rathke vesicle). Postnatally, the cleft usually becomes reduced to a number of larger and smaller cysts (Rathke's cysts) lined by a ciliated epithelium and containing a yellowish, colloidal material. With age, an increasing number of basophils of the pars intermedia cross the borderline between adeno- and neurohypophysis and "invade" the posterior lobe, where they are found in close proximity to the nerve-fiber endings and the capillaries.

Light microscopy reveals only slight differences between the usually polygonal cells of the pars intermedia and the basophils of the anterior lobe. Their granules stain specifically with the periodic-acid-Schiff (PAS) reagents as well as with aldehyde-fuchsin and resorcin-fuchsin. Electron microscopy shows that these granules are characteristically accumulated close to the basal lamina. Their diameter varies between 2000 and 3000 Å. The melanocyte-stimulating hormone (MSH) produced by these basophils is a polypeptide which seems to be

bound to a glycoprotein within the granules. From the pituitaries of humans and other mammals, two different melanocyte-stimulating hormones have been isolated up to now. The sequential arrangement of the amino acids in both the strongly basic alpha-MSH and the slightly acid beta-MSH is similar to that found in the adrenocorticotropic hormone ACTH over large parts of the molecule. In the amphibian skin, MSH causes a dispersion of the melanin granules in the chromatophores and therefore a darkening of the skin. In mammals, MSH is assumed to stimulate the melanin production in the melanocytes.

The pars intermedia of the adenohypophysis and the adjacent portions of the neural lobe form the so-called distal adeno-neurohypophyseal contact zone, where blood vessels and nerve fibers are characterized by a specific arrangement (see p. 82).

Pars Infundibularis or Tuberalis. The pars infundibularis of the adenohypophysis forms a sleeve around the infundibulum, from which it is separated by a thin sheet of connective tissue continuous with the pia mater. Its thickest portion (approximately 40 to 80 μ) is on the anterior surface of the neural stalk, whereas the posterior portion is often incomplete or absent. The pars infundibularis is traversed by the major arterial vessels and the hypothalamo-adenohypophyseal portal venules, both extending to the anterior lobe. The longitudinally arranged cords of epithelial cells occupy the interstices between the blood vessels. A network of fine reticular fibers separates these two elements. Pars tuberalis, blood vessels and neural stalk together form the so-called proximal adeno-neurohypophyseal contact zone which is discussed below.

The epithelial cords of the pars tuberalis contain only a few acidophils and basophils, but predominantly undifferentiated, cuboidal cells resembling the chromophobes of the anterior lobe (pars distalis). Characteristically, these cells measure some 15 μ in diameter and contain many small, rod-shaped mitochondria. The Golgi apparatus is less developed than that of the cells of the glandular lobe. In the cytoplasm, numerous small lipid and colloid droplets are often present, as well as large amounts of glycogen. Usually, some pars infundibularis cells are found to be arranged in follicles similar to those of the pars intermedia.

4. The Hypothalamo-Adenohypophyseal Connections

In contrast to the posterior (neural) lobe, which must be regarded as a specially differentiated subdivision of the diencephalon, the anterior lobe is a true endocrine gland, i.e. it produces a number of hormones itself. The hypothalamus controls the incretory activity of the glandular lobe not through nerve fibers reaching the endocrine cells, but through the release of specific substances (neurohormones) into a unique capillary network in the hypophyseal stalk. This vascular system, which combines the diencephalon and the adenohypophysis to a functional unit, is established very early. In the human embryo 10–11 mm in length, the pars infundibularis of the adenohypophysis is already present in the form of two epithelial buds at the anterior surface of the adenohypophyseal vesicle (see p. 77). These two buds grow rapidly upward to the floor of the diencephalon and also contact the infundibulum which extends ventrally. At the same time, a dense network of blood vessels develops and surrounds the hypophysis. This network is supplied by both the meningeal plexus of the embryonic brain anlage and the hypophyseal arteries arising from the internal carotids. The two inferior hypophyseal arteries arborize within the organ capsule, branches extending to the neural and (to a lesser extent) also to the glandular lobe.

The superior hypophyseal arteries anastomose freely in the region of the root of the hypophyseal stalk. From this network, a number of capillaries form characteristic loops which extend to varying depths of the nervous substance of the median eminence. The afferent limbs of these capillaries which comprise the so-called *primary plexus* have a much smaller diameter than the efferent limbs of the loops draining into the hypophyseal *portal veins*. These veins can be recognized with the naked eye. They run downward around the hypophyseal stalk (mainly at the anterior surface of the pars infundibularis of the adenohypophysis) and supply the sinusoids of the anterior lobe.

Within the median eminence, the capillary loops of the primary plexus are found in close relation to nerve-fiber endings of the *tubero-hypophyseal tract*. On both sides of the infundibular recess, the pericarya of these axons constitute the nucleus tuberis infundibularis, the nucleus hypothalamicus dorsomedialis, and the nucleus ventromedialis. These nuclear masses are not only characterized by their proximity to the hypophyseal stalk, but also by the morphological similarity of their neurons, which are small and rather poor in cytoplasm. In contrast to the fibers of the hypothalamo-neurohypophyseal tract, the neurons of the tubero-hypophyseal tract do not react with the Gomori stain. Their secretory activity has,

however, been confirmed by numerous ultrastructural studies. The neurohormones produced by these cells are carried along the axons and released into the narrow intercellular space separating the nerve-fiber endings from the fenestrated endothelium of the primary capillary plexus. These hormones pass through the hypophyseal portal venules to reach sinusoids of the anterior lobe where they *stimulate or inhibit* the production and release of the specific adenohypophyseal hormones (see also Chap. II, p. 28). Thus, through a singular process of vascularization which in the human is fully developed only at the end of intra-uterine life, the anterior lobe becomes functionally (and indirectly) connected to the center controlling most endocrine functions of the body, the hypothalamus.

In contrast to the pars infundibularis, in which the *proximal* adenoneurohypophyseal contact zone is formed by the primary capillary plexus, the pars intermedia, as the *distal* contact zone between anterior and posterior lobe, lacks such special blood vessels. In this region, however, another kind of interaction between adeno- and neurohypophyseal elements appears to become increasingly important with advancing age, although its functional significance is by no means certain. With the disappearance of the initial cleft (see p. 80), basophilic cells of the pars intermedia frequently extend a considerable distance into the parenchyma of the posterior lobe where they are found in close spatial relation to both the axon endings of the supraoptic-neurohypophyseal tract and the sinusoidal capillaries. The nerve fibers surrounded in this way by adenohypophyseal elements appear to be particularly rich in neurosecretory material. The hypothalamo-neurohypophyseal tract thus contacts not only the vascular system of the neural lobe (as well as the ventricle system of the diencephalon) but also specific cells of the glandular lobe. Experiments have shown that prolonged thirst leads not only to depletion of the posterior lobe in neurosecretory material, but also to atrophy of the pars intermedia. On the other hand, interruption of the hypothalamo-neurohypophyseal tract was found to result in a marked hypertrophy of the pars intermedia. In spite of the morphological differences, a functional analogy between the proximal and distal adeno-neurohypophyseal contact zones might therefore be postulated.

C. Chemistry and Biochemistry

Seven different adenohypophyseal hormones have been definitely determined so far (Table 1). Five of these hormones are adenotropic (see below), i.e. hormones regulating other endocrine glands. Two of these adenotropic hormones are gonadotropins controlling gonadal function in males and females.

Gonadotropins, adrenocorticotropin and thyrotropin are discussed with reference to their target gland in the appropriate chapter. For prolaction see also Chap. XI, p. 674f. It is still unknown what physiological significance is attributable to MSH, the melanocyte-stimulating hormone, and EPS, the exophthalmos-producing substance.

1. Prolactin

Prolactin, otherwise known as mammotrophic hormone, lactogenic hormone, or LTH (luteotrophic hormone) has a wide variety of functions in the reproductive system throughout the animal kingdom from the fishes to the primates (SHERWOOD, 1971; NICOLL, 1972). In mammals, luteotrophic activity has been demonstrated in rodents but not in primates, and it is therefore no longer included in the group of gonadotropins. Its classic function in mammals, including man, is stimulation of lactation, so that the name most commonly used is prolactin. Although prolactin has been extracted and purified from the pituitaries of a large number of species and the amino-acid sequence of ovine prolactin has been determined (LI, 1969) the controversy as to whether prolactin and growth hormone are two different hormones in the human went on for some time, a problem complicated by the fact that pure human

Table 1. Hormones of the human anterior pituitary

Hormone	Chemistry	Isolated	Constitution known	Synthesis	Molecular weight
ACTH	Polypeptide	+	+	+	4540
β-MSH	Polypeptide	+	+	+	2660
Growth hormone	Polypeptide	+	+	+	21500
TSH	Glycoprotein	(+)	−	−	About 25000
FSH	Glycoprotein	Partly	−	−	About 30000
LH	Glycoprotein	Partly	−	−	About 26000
Prolactin	Polypeptide	+	+	−	23300 (ovine)

growth hormone also has some intrinsic lactogenic activity. Clinical observations of milk production in growth hormone-deficient women and a variety of biologic, immunologic, and chemical studies have now proved there is a difference between these two hormones in man. Prolactin can now be determined radioimmunologically and does not cross-react with human growth hormone. Besides its action on the mammary gland, it also has effects on the testes (BARTKE, 1970), and possibly on water and electrolyte transfer in the kidneys (MANKU, 1972) and intestine (RAMSEY, 1972). There seems to be a reciprocal mechanism controlling secretion of prolactin and gonadotropins. A potent releasing stimulus is suckling in nursing mothers. Phenothiazines, TRH stress and hypoglycemia increase prolactin secretion, whereas α-dopa, 2-α-Br-ergocryptine suppress it. Renal failure is frequently associated with high plasma prolactin, estrogens cause a rise in adults. Using a sensitive radioimmunoassay (HWANG, 1971) on the base of human prolactin, normal values for plasma in women are in the range of 10 ng/ml, for men a little bit less. Dependence of breast cancer from prolactin is discussed (SALIH, 1972; BOYNS, 1972; FRIESEN, 1973).

2. Growth Hormone

Growth hormone (somatotropin, STH) is the only pituitary hormone which exerts a direct effect without the involvement of another endocrine gland. A growth hormone-like factor has been detected which is produced, curiously enough, in a tapeworm of mice and has practically all biological effects of growth hormone except the lipolytic effect. It seems to be of high molecular weight and immunochemically different from growth hormone (Sparganum growth factor, STEELMAN, 1971), and different from the sulfation factor or somatomedin.

Apart from growth hormone, the relevance of other metabolic hormones [adipokinin, lipotropin (LEVINE, 1964; LUFT, 1966; ANSELMINO, 1965; RUDMAN, 1965)], and pancreatotropic hormones is still uncertain.

a) Chemistry

Growth hormone, (somatotropin, somatotropic hormone, STH), is a highly species-specific polypeptide hormone. The growth hormones of different mammalian species differ chemically, immunologically and in their biologic action.

Human growth hormone (HGH) consists of an unbranched chain of 188 amino acids with 2 disulfide bridges and has a molecular weight of 21,500 at an isoelectric point of pH 4.9. The complete amino-acid sequence has been successfully determined (LI, 1966) and it has recently been synthesized (LI, 1971).

Human growth hormone is extracted from human pituitaries obtained at autopsies either by using hot glacial acetic acid, purifying with oxycellulose and precipitating with ethyl alcohol (RABEN, 1959), or by using phosphate buffer precipitation with ammonium sulfate at different pH, resin, and sephadex chromatography (LI, 1966; ROOS, 1963). Growth hormone is stored in large amounts in the hypophysis and forms 3% of the acetone dry powder of the hypophysis. One human hypophysis will therefore yield 3–5 mg or even more (GEMZELL, 1958). The content of growth hormone in the hypophysis seems to be independent of the age of the individual.

Careful treatment with proteolytic ferments causes partial decomposition without affecting the biological action. Chymotrypsin can break down human growth hormone by 25%, and pepsin can break it down to 50% without affecting its potency. Therefore, the "active core" probably contains a peptide nucleus of only about 100 amino acids. Despite crystallization the human growth hormone currently extracted does not appear to be fully homogeneous. It can be dispersed into four active components by starch-gel electrophoresis and also by sephadex chromatography. It is possible that artifacts producing subtle changes occur during extraction. The immunological behavior of the hormones is similar, but they differ in biologic potency. In acromegaly, growth hormone is radioimmunologically high, and prolactin is found to be low. In the lactating woman, however, the situation is reversed, and growth hormone is found to be normal, whereas prolactin is high (ROTH, 1967).

Growth hormones of other mammalian species have other amino-acid sequences and molecular weights up to 48000. Bovine and ovine growth hormones probably consist of branched polypeptide chains. Similarities in the molecular structure exist between the growth hormones of the human and monkey, between those of pig and whale, and those of cow and sheep.

Human growth hormone produces antibodies in the rabbit which only react with human or monkey growth hormone. Bovine growth hormone forms antibodies which precipitate bovine and ovine growth hormones in agar. Bovine growth hormone is not effective in man, monkey, or guinea pig. On the other hand, growth hormone from man and from monkeys is effective in all vertebrates with the exception

of guinea pig and fish. The effect of human growth hormone in the rat, however, decreases after 10 days because of antibody formation, whereas bovine growth hormone continues to be effective.

b) Plasma Concentration, Half-Life, Turnover, Daily Production

Normal serum growth hormone values are 0–3 µg/ml (>90%) when measured by radioimmunological methods (HUNTER, 1964; UTIGER, 1964; GLICK, 1965; BODEN, 1967) under standard conditions in the adult following an overnight fast.

Age-dependence: Considerably elevated values are found in premature infants. The values estimated from the umbilical venous blood in newborns are also high. In infants and babies they lie between 0 and 10 µg/ml until the fourth year of age. These findings indicate that the notion that growth during the first year of life is not affected by growth hormone is mistaken. The values are between 0 and 5 µg/ml from the fourth year onwards, and no differences are demonstrable in the basal values from early adulthood to old age. Growth hormone secretion increases during sleep, but paradoxical sleep inhibits its secretion (HONDA, 1969). Sexual differences have been demonstrated (MERIMÉE, 1966). Oral contraceptives are thought to increase the level of growth hormone by approximately 10 µg/ml (SPELLACY, 1967).

The half-life of human growth hormone is 25 minutes. The fractional turnover rate is 2.8% or 0.5–0.8 mg/day under basal conditions. The total daily secretion under the influence of food, starvation, and muscular activity must lie between 3–5 mg, according to the therapeutic dose for pituitary dwarfism (PARKER, 1962; GLICK, 1965). The multi-exponential curve of decrease causes uncertainty in the calculation of other compartments and of the half-life (CAMERON, 1969; TAYLOR, 1969). According to more recent studies based on the determination of integrated plasma growth hormone levels (KOWARSKI, 1971), the daily secretion of growth hormone is even lower and ranges from 0.6 to 1.5 mg per day, being higher in women than in men. Breakdown is achieved partly by proteolytic enzymes not specific to growth hormone alone and partly by enzymes which disintegrate the disulfide linkage (PARKER, 1962; SIREK, 1964). A small proportion has been recently demonstrated to be excreted unchanged in the urine.

Human growth hormone does not pass into the placenta; consequently, the high concentration of growth hormone in the blood of the newborn comes from the infant. The half-life of the maternal growth hormone is reduced before birth (LARON, 1966).

3. Substances with Partial Effects of Growth Hormone

a) Lipolytic Polypeptides from the Pituitary

Apart from growth hormone there are other hormones secreted by the adenohypophysis which have a lipolytic action *in vitro*, partly species-specific. These are ACTH, TSH, and the α- and β-MSH. Arginine-vasopressin, one of the neurohypophyseal hormones, also has a lipolytic effect. The physiological significance of the lipolytic activity of these hormones is unknown.

In addition, other proteins and polypeptides with a lipolytic effect have been isolated from the pituitary gland of different types of animals (SCHWANDT, 1968; TRYGSTAD, 1967/1968; RUDMAN, 1962/1965). Although a hypophyseal hormone concerned specifically with fat metabolism was discovered over 30 years ago (ANSELMINO and HOFFMANN, 1965), it is still not known whether such substances (lipotropin, adipokinin) are involved in regulating fat metabolism, with the exception of growth hormone.

A substance of protein character can be demonstrated in the urine of fasting persons. This substance mobilizes free fatty acids *in vitro* from the adipose tissue of the rat, and *in vivo* it produces lipemia, fatty degeneration of the liver, and ketosis. The occurrence of this substance is dependent on an intact hypophysis. It is not claimed to be identical to growth hormone or ACTH (CHALMERS, 1960).

b) Chorionic Growth Hormone-Prolactin Complex (CGP, HPL, see also p. 677*)*

Pregnancy leads to changes in the facial features which can, however, vary individually and which are reminiscent of acromegaly (acromegaloidism of pregnancy). Pregnancy also causes growth of the breasts and an anabolic metabolic state. These three changes cannot be explained by an increase of the sex hormones alone during pregnancy, so that it is likely that the activity of growth hormone is also increased during pregnancy. On the other hand, hypophysectomy during pregnancy does not impair the course of the pregnancy, and the physical changes in the woman do not regress.

A protein has been extracted from placentas of the human and the monkey. In animal experiments, it shows activities similar to those

of prolactin and of growth hormone also, but only to a very slight degree. It reacts immunologically with antiserum to human growth hormone, again more weakly than human growth hormone does. The biochemistry, physiology, and clinical significance of this chorionic growth hormone-prolactin (KAPLAN, 1964) or the placental lactogen (JOSIMOWITSCH, 1962) which has been demonstrated in the serum and urine of pregnant women, as well as in the retroplacental blood, is discussed in Chap. XI, p. 677.

c) Hypoglycemic Factor

Tests are still necessary to determine whether the small molecular polypeptide obtained from bovine growth hormone with an exclusively hypoglycemic action is a hormone distinct from growth hormone (HUGGINGS, 1961).

d) Bovine Growth Hormone Digests

It has apparently been confirmed that bovine growth hormone treated carefully and briefly with trypsin can produce pronounced metabolic activities of human growth hormone in people with hypopituitarism. This suggests that there is an active core common to both molecules (SONENBERG, 1967; NADLER, 1967).

D. Physiology

1. Metabolic Effects of Growth Hormone

Growth hormone is a metabolic hormone playing a definite role in the regulation of protein, carbohydrate, and fat metabolism. It also appears to have the effect of a biological synergist which can intensify certain actions of other hormones, such as LH, testosterone, and ACTH (in relation to growth of the adrenals). Growth hormone and insulin form an important synergistic and antagonistic system.

The effect on organs such as the liver and the kidney is demonstrated by the increase of bromsulphalein-, insulin-, creatinine-, and PAH-clearances.

a) Protein Metabolism

STH has an anabolic effect. It promotes protein synthesis, and under its influence, the nitrogen balance becomes positive. The urinary excretion of urea, creatine, and nitrogen decreases, with a relative increase in the nitrogen of the amino acids. Plasma urea and nonprotein nitrogen fall, and certain amino acids (Tre, Ser, Gly, Meth) increase (ZACHMANN, 1968). A single intramuscular injection of 2 mg is sufficient to produce an effect of this nature lasting for 2–3 days in the adult.

It has been shown *in vitro* and *in vivo* that growth hormone promotes the transport of specific amino acids, particularly leucine and glycine, into the cells (KNOBIL, 1961). This transport activity is not inhibited by puromycin and is therefore independent of protein synthesis. In addition to this transport activity the mode of action of growth hormone on protein synthesis can be investigated in the cell-free system of the rat liver. In this cell-free system a growth hormone administered to the animal *in vivo* promotes the incorporation of amino acids into protein. This does not affect the amino acids activating enzymes, or the uptake, or the transmission of the soluble RNA. On the other hand, growth hormone promotes the linking together of activated amino acids into polypeptides within the microsomes (KORNER, 1965). It influences the synthesis of the Transfer-RNA, which is the limiting step in protein synthesis, and therefore increases the number of ribosomes and their aggregation to form polysomes (TALWAR, 1964; KORNER, 1968). These activities of growth hormone are not inhibited by actinomycin, and they do not, therefore, take place via DNA metabolism. Cell division may be stimulated under the influence of growth hormone (CATER, 1957; MOON, 1962), but the frequent mitoses are more likely to be due to the increased supply of substrates.

Although a direct action of growth hormone on protein synthesis has been demonstrated *in vitro*, synergism with insulin is essential to produce the maximum anabolic effect. Insulin alone also promotes the introduction of amino acids into the cells, but it has no growth-promoting effect after hypophysectomy.

In vitro, growth hormone does not promote the incorporation of sulfur into cartilagenous tissues as does the sulfation factor. This factor, however, increases in the serum under the influence of growth hormone. Therefore, growth hormone probably has an indirect effect upon growth through generation of the sulfation factor (DAUGHADAY, 1966). It has recently been shown that the sulfation factor is probably identical to NSILA-S (see Chap. XIII, p. 749f.) and it has been tremed somatomedin (see p. 88).

b) Carbohydrate and Fat Metabolism

In vitro, a lipolytic effect of relatively high doses of growth hormone can be demonstrated in isolated fat tissue due to the hydrolysis of

triglycerides in the depot fat (RABEN, 1962; WINEGRAD, 1959). Free fatty acids and glycerol are released into the medium, the latter to a greater extent, since intensive intracellular re-esterification of the free fatty acids occurs at once. The intracellular glycerol requirements are covered by increased glucose uptake by the tissue or by glycogenolysis. While glucose taken up under insulin stimulation is broken down via the pentose phosphate pathway, glucose taken up under the influence of growth hormone is metabolized by glycolysis and the KREB'S cycle. Recently, lipolysis produced by physiological concentrations of growth hormone in the presence of added glucocorticoids has been successfully demonstrated in isolated fat cells (FAIN, 1965). It is not clear how lipolysis is induced by growth hormone. It begins after a latent period of 1–2 hours and reaches a maximum after 4 hours. It is inhibited by actinomycin D (FAIN, 1965). Tissue lipase is not activated as is the case with adrenaline or ACTH. *In-vitro* experiments on the effects of growth hormone on muscular tissue have not produced consistent results (BODEL, 1962; HENDERSON, 1961).

In vivo, 3 phases can be differentiated among the metabolic effects of growth hormone. These are: an early phase lasting 0–30 minutes, an intermediate phase lasting between 30–240 minutes, and a late phase lasting some days or weeks.

In the first phase, human growth hormone promotes the intake of free fatty acids and glucose into the musculature. A brief fall in the free fatty acids and blood glucose occurs simultaneously during the first phase, particularly in the hypophysectomized or adrenalectomized animal. The hypoglycemia is difficult to explain. It is not caused by a rise in insulin, since the plasma insulin level remains low (ZAHND, 1960). Increased transport of glucose into fat tissue under the influence of lipolysis and re-esterification has been considered (WEIL, 1965), but decreased glucose uptake by the musculature and fatty tissues precedes lipolysis. Protein synthesis, which removes glucoplastic amino acids from gluconeogenesis, has also been considered as the cause of hypoglycemia, but protein synthesis commences only after several hours. The possibility that the early hypoglycemia due to growth hormone may be caused by carbohydrate retention in the liver has been discussed. Today, the role of somatomedin has to be considered.

In the second phase, growth hormone promotes lipolysis in adipose tissue, with the release of glycerol and free fatty acids into the blood and an increase in their blood level. The increased flux of free fatty acids exceeds the capacity of the liver to oxidize them completely, so that they leave the liver as ketone bodies. Peripheral tissues cannot cope with these, and thus keto-acidosis may develop under the influence of growth hormone. Glucose uptake by the musculature is inhibited, glucose tolerance decreases, and the assimilation coefficient falls to sub-normal values (IKKOS, 1962). The increased supply of free fatty acids is suspected to inhibit glucose uptake (RANDLE, 1964). However, growth hormone reduces glucose absorption before the free fatty acids in the blood rise. It is possible that inhibition of glucose absorption by the musculature and fatty tissues is the primary effect of growth hormone, and that lipolysis only results from a carbohydrate deficiency in the fatty tissue. Gluconeogenesis in the liver is increased under the influence of free fatty acids (SÖLING, 1966), which causes a further fall in the glucose tolerance. When insulin and growth hormone act at the same time, the increased glucose absorption induced by insulin is diminished, and the lipolytic effect of growth hormone is blocked.

Third phase and growth hormone-diabetes: Growth hormone promotes protein synthesis and growth only in the presence of insulin. An increased secretion of insulin, "an extra-insulin", is not necessary, as was indicated by earlier results, although growth hormone inhibits glucose absorption in the musculature and fatty tissue and reduces the sensitivity to insulin (SCOW, 1960). When growth hormone is administered for 4 days to 2 weeks, the insulin secretion diminishes in carnivores, such as dog and cat; the blood sugar rises, and reversible "idiohypophyseal" diabetes develops. The B-cells of the pancreas are irreversibly damaged if the administration of growth hormone is further continued, and permanent "metahypophyseal" diabetes indistinguishable from that produced after pancreatectomy develops. The hyperglycemia is accompanied by keto acidosis, glucosuria, and acetonuria.

Man and monkey react in a similar way to dog and cat (IKKOS, 1962). In herbivorous animals, such as rabbit, guinea pigs, and sheep, whose islet cells are more resistant and are capable of regeneration, idiohypophyseal or methahypophyseal diabetes can only be produced by concurrent forced feeding, or partial pancreatectomy, or by previous treatment with alloxan.

Growth hormone always produces growth in the rat, where the epiphyseal cartilages remain open throughout life. Puppies and cats grow under the influence of growth hormone as long as the epiphyseal cartilages remain open. In

adult animals, however, diabetes develops, since the glucose can no longer be used for building tissues. Puppies treated for long periods with growth hormone fail to grow and become diabetic. Administration of insulin abolishes the diabetes and re-establishes growth. Growth hormone only has a diabetic action when growth is impossible or when the insulin production is limited and the glucose cannot be used in other ways. Lactating or pregnant rats grow. If lactation in the goat is prevented by resection of the udder, diabetes develops (BERT, 1883).

Although 10 mg HGH i.m. reduces the glucose tolerance after 12 hours in the normal human (MITCHELL, 1970), only 25% of patients with active acromegaly are diabetic. Contrary to previous suspicions, diabetics do *not* have raised growth hormone levels in the plasma (GLICK, 1965). Growth hormone levels can, however, be relatively high in relation to the raised blood sugar in diabetics, but this is due rather to impaired intracellular carbohydrate utilization in the regulation centers (see p. 761) which may possibly be dependent upon insulin (GLICK, 1965). Whether growth hormone is or is not connected with diabetic retinopathy is still under discussion (POWELL, 1966; LUNDBAEK, 1970).

c) Effect of Growth Hormone on Energy Balance

Insulin and somatotropin regulate liberation, utilization, and regeneration of glucose, free fatty acids, and amino acids in the sequence of food intake or fasting.

The blood sugar rises immediately after intake of food and leads to the release of insulin after 1 hour and to a fall of growth hormone in the blood. Glucose absorption and glycogen synthesis are promoted. At the same time, triglyceride synthesis starts, lipolysis is blocked, and the free fatty acids in the blood fall. In the second phase, the glucose falls and growth hormone begins to rise in the presence of continuous insulin activity. This constellation promotes protein synthesis to a maximum while glucose absorption decreases with reduced glucose supply, thereby reserving the glucose for the nervous system. Finally, in the third phase, in the fasting or starving state the blood sugar falls to normal values and insulin falls while growth hormone continues to rise, inducing lipolysis. Glucose absorption is then at a minimum, protein synthesis is at a standstill without insulin, and free fatty acids are disposed of by the musculature. The glucose requirement during this phase is not only reduced by the supply of free fatty acids, but also particularly by glycerol, which is a precursor of glucose. Growth hormone fulfills different important functions for the period without any nutritional intake. In this way, it reduces the glucose requirement in the musculature, abates protein synthesis at the same time, and offers the musculature and liver free fatty acids in return, so that the remaining glucose is at the disposal of the nervous tissue which is dependent on glucose (ZIERLER, 1963).

Some authors still doubt that so many different types of actions, such as protein synthesis, fat metabolism, insulin synergism and antagonism, growth, and production of diabetes, can be due to the same hormone. Two pituitary hormones, somatotropin and adipokinin, chemically similar and with the same immunological behavior but with different biological activity, have been considered (LEVINE, 1964; LUFT, 1966). Both, however, like the purest growth hormone preparations so far known, produce protein synthesis and lipolysis. In addition, the connection of the anabolic effect with the mobilization of fuel from their depots seems to be rational. The chief effect of somatotropin is not so greatly cerned with growth (growth hormone is produced in the same amounts after growth is completed) but is concerned rather with the maintenance of homeostasis in catabolic states, such as fasting, muscular activity, and hypoglycemia. The difficulty in accepting that the same substance regulates acute metabolism and the long-term process of growth may be overcome by regarding the effect on growth as being indirect (sulfation factor) and not dependent of short-lived variations (DAUGHADAY, 1966).

d) Electrolyte and Water Metabolism

The urinary excretion of sodium, potassium, phosphorus, and chloride decreases under the influence of growth hormone, whereas the urinary calcium excretion generally rises. Aldosterone secretion is not directly influenced. Water retention is parallel to that of sodium, and the extracellular space increases (BIGLIERI, 1961). The calcium and phosphorus balance is generally positive, although a negative calcium balance has occasionally been observed (KNOBIL, 1964). Intestinal calcium absorption seems to be enhanced, and calcium may possibly be mobilized from the skeleton. This may be due to an indirect parathyroid activation (FRASER, 1960). Phosphorus and calcium are stored in proportion to the nitrogen, corresponding to the composition of muscular tissue. The excess potassium retained probably goes into

the liver. Among the serum electrolytes, only phosphorus retention is reflected in an elevated phosphate value.

e) Effects on Other Endocrine Glands

α) *Pancreas:* Contrary to previous opinions, there has been no successful demonstration that physiological amounts of growth hormone can directly induce the formation or release of insulin from the pancreas. Consequently, growth hormone leads only indirectly to increased insulin secretion and hyperplasia of the β-cells.

β) *Adrenal glands:* Enlargement of the adrenals and the thyroid gland under growth hormone is due to the effect on growth. There is no change in the excretion of 17-ketosteroids, 17-hydroxysteroids or aldosterone.

γ) *Thyroid gland:* An increase in the BMR is due to extrathyroidal factors. The protein-bound plasma iodine or thyroxine is not influenced. On the other hand, radioiodine studies have demonstrated an elevated velocity index in acromegaly (2/48 h uptake ratio) corresponding to an accelerated iodine turnover.

δ) *Parathyroids:* These glands can be activated indirectly through an elevated serum phosphate level and a fall in the calcium level.

For effects on milk secretion see p. 695. For discussion of the effects of other hormones on the secretion of growth hormone see p. 90f.

f) Influence on Growth (See also Chap. XIX)

In pituitary insufficiency in animals and man growth is interrupted in the growing phase even when nutrition remains undisturbed. Growth hormone re-establishes normal growth. It works directly on the tissues without involving other endocrine glands, since it is also effective in eviscerated animals providing that small amounts of insulin and probably "somatomedin" (see below) are present. In animals where the epiphyseal cartilages are open throughout life, STH can produce continuous growth for the rest of their lives when the dosage is adjusted to the increasing weight. Thus, in 435 days a gigantism of over 600 g could be produced in rats, with the experiment only coming to an end when the maximum life-span of the rat was reached.

Growth is presented first as an increase in weight due to synthesis of protein and water retention corresponding to the composition of the embryonic tissue. The fat content of the entire organism decreases, and growth of the liver, kidneys, heart, stomach, and gut is in proportion to body weight. Compensatory hypertrophy of the remaining kidney after unilateral nephrectomy is only possible under the effect of growth hormone (ASTARABADI, 1963).

STH promotes endochondral bone growth. Under its influence columns of cartilage and lamellae of bone with numerous osteoblasts are formed in the epiphyseal cartilages. Increased serum alkaline phosphatase reflects osteoblastic activity. The cells of the epiphyseal cartilages increase in number and size. Measurement of the width of the columnar cartilage at the transition to the bony rib has been used as a test for the activity of growth hormone (see p. 124). The incorporation of sulfate into cartilage is increased and probably occurs indirectly through the sulfation factor (see p. 89). In addition to polysaccharide synthesis, soluble collagen is also formed and its rapid turnover is apparent in the appearance of more hydroxyproline in the urine. Growth hormone promotes growth even in the absence of thyroid hormone. Thyroid hormone, however, can intensify the growth activity of STH without itself influencing growth. On the other hand, insulin is necessary for growth, even if it does not promote growth by itself. The effect on growth and that on carbohydrate and fat metabolism are probably due to the same hormone, and the connection of growth with economy of carbohydrates and amino acids at the expense of fats is reasonable.

It is not known why growth hormone can cause growth in certain phases of life and has no effect in other phases. The capacity of the tissues to respond must vary in the different phases of growth. It is possible that the effect of growth hormone on growth is only permissive.

g) Sulfation Factor, Somatomedin, NSILA-S

A peptide with a molecular weight of approximately 6000–10000 which increases the incorporation of ^{35}S and H^3-thymidine into rat and chicken cartilage has been partially purified from serum. This substance has been called "sulfation factor", and sometimes "thymidine factor". It was established that patients with acromegaly have high concentrations of sulfation factor in the serum, whereas pituitary dwarfs do not; when pituitary dwarfs are treated with growth hormone the "sulfation" activity in their serum increases. Some investigators believe that the sulfation factor is produced in the body, possibly in the liver, under the influence of growth hormone. The hypothesis has been put forward that growth hormone by itself does not stimulate growth but rather that its effects are mediated by the sulfation factor. It has therefore been suggested that the sulfation factor be called "somatomedin".

Nonsuppressible insulin-like activity (NSILA-S) has proven extremely active in stimulating the incorporation of ^{35}S into rat cartilage and the growth of fibroblasts in culture. In all likelihood NSILA-S and somatomedin are identical. However, the physiological significance of somatomedin and NSILA-S must be interpreted with caution. There are probably several substances in the blood which have some growth-promoting activity, and growth hormone is not the only hormone controlling their concentration in the serum.

2. Regulation of the Secretion of Growth Hormone

Growth hormone, as an important metabolic regulator, shows considerable variations in concentration during the course of the day. There are, however, short bursts during night sleep, whose nature has not yet been explained. It is now known that the following conditions affect the release of growth hormone:

a) Hypoglycemia and fall in blood sugar
b) Fasting
c) Muscular activity
d) Certain amino acids
e) Stress: fever, operations
f) Catecholamines
g) Other mechanisms.

a) Hypoglycemia

A fall in the blood sugar to 50% of its original value causes an approximately 5-fold increase of growth hormone. The hormone level first falls for a short interval of time, then increases to the maximum between 15–30 minutes after the nadir of the blood sugar is reached and persists at this level for several hours. The hypoglycemia can be released by insulin or tolbutamide, or by blocking liver gluconeogenesis. The increase in growth hormone can be prevented by giving glucose at the same time. On the other hand, hypoglycemia due to alcohol produces no rise in the growth hormone, probably because the hypoglycemia develops slowly. Growth hormone secretion persists at the same level throughout the hypoglycemia. Since the half-life of growth hormone is 20–30 minutes, there must be continuous secretion of the hormone to maintain a constant level. A rise in the growth hormone may even be produced by slight decreases of 10 mg/100 ml in the blood sugar which is a physiological occurrence during the day (LUFT, 1966).

The releasing stimulus is obviously a deficiency of the intracellular utilizable glucose, since administration of 2-deoxyglucose also leads to a rise in the growth hormone in spite of hyperglycemia.

A fall in blood sugar also causes release of growth hormone even if the blood sugar values are still above normal. Variable increases in growth hormone not proportional to the degree or speed of the fall in blood sugar can arise 4–6 hours after glucose overloading without necessarily producing clinical symptoms due to the fall in blood sugar.

Testosterone promotes and medroxyprogesterone inhibits the increase of growth hormone after hypoglycemia (ILLIG, 1970).

b) Fasting

A fairly long fast, i.e. deprivation of food for more than 12–15 hours, leads to a continuous rise in growth hormone in the blood. There is usually an initial high peak, after which the concentration varies around the average value during subsequent days if fasting is continued. The rise is not so pronounced or rapid as in hypoglycemia and this increase may be absent or much less pronounced in the obese. Nevertheless, overweight subjects can break down their fat depots just as well during fasting and their metabolism is not maintained at the expense of protein. The failure of growth hormone to rise is a result rather than the cause of obesity.

c) Muscular Activity

Moderate muscular activity such as walking for 30 minutes causes a pronounced rise of growth hormone levels in the blood. It has been suggested that this rise is greater in women and in men treated with estrogens (FRANTZ, 1965). It can be mitigated considerably by previous administration of glucose. Wide variations of short duration result from continuous activity (HUNTER, 1966).

d) Amino Acids

Infusion of 15–30 g of certain acids over 30 minutes leads to a considerable rise (5- to 10-fold) in the plasma growth hormone after 1–2 hours. This effect is independent of the blood sugar. There is a simultaneous release of insulin, and the two act synergistically to enhance protein synthesis. Arginine, ornithine, histidine, and lysine are effective; phenyalanine, methionine, valine, and threonine are feebly effective; leucine is not always active; and isoleucine has no effect (KNOPF, 1965; FAJANS, 1967; RABINOWITZ, 1966). It has been suggested

that the two sexes respond differently and that estrogens promote the response (MERIMÉE, 1967).

e) Operations

Serious operations involving the opening of the abdomen or the thoracic cavity lead to a considerable rise of growth hormone which cannot be inhibited by continual glucose infusions or even by hyperglycemia. Anesthesia alone or with electric shock does not produce the release of growth hormone. Minor surgical procedures, such as herniotomy, cause only a slight increase in growth hormone, which can be prevented by glucose administration.

f) Catecholamines

Adrenaline stimulates the release of growth hormone, probably via cyclic AMP (GAGLIARDINO, 1968). The α-receptors appear to promote growth hormone release by hypoglycemia, whereas the β-receptors have an inhibitory effect. Promotion and inhibition can be abolished by phentolamine (BLACKARD, 1968) respectively propranolol (IMURA, 1968).

g) Other Mechanisms

Besides the stimuli for growth hormone secretion mentioned above, other compounds such as α-MSH, L-Dopa, and metyrapone have been shown to cause a substantial increase in the plasma levels of growth hormone.

h) Stimuli Decreasing Growth Hormone

Administration of glucose causes an elevated growth hormone level to fall in healthy subjects and in diabetics. The effect of protein administration is not consistent (amino acids, see p. 89). Overloading with fat has no effect on the release of growth hormone. Glucocorticoids inhibit the release caused by hypoglycemia (PECILE, 1966) and the normal diurnal variation (STIEL, 1970).

i) Localization of the Regulation

Stress and metabolic effects are registered and co-ordinated with endogenous rhythms (sleep) in the hypothalamus which then releases GRH (growth hormone releasing hormone) to regulate the discharge of growth hormone from the adenohypophysis (see Chap. II, p. 32). Normal basal values are still found after section of the pituitary stalk. There is no rise with hypoglycemia following lesions of the ventromedial nucleus in the hypothalamus. In the monkey the hypoglycemic stimulus is ineffective. Growth hormone releasing hormone (GRH see Chap. II, p. 32), which leads to the release of growth hormone from the adenohypophysis *in vitro* and *in vivo*, has been extracted from the hypothalamus*. It is not known how the stimulus is registered in the hypothalamus. Intracellular carbohydrate deficiency seems to be a stimulus, but this does not explain the effect of fasting since the intracellular carbohydrate content of the central nervous system does not decrease and hypoglycemia does not develop.

It is not known how the hypothalamus registers muscular activity. Different factors, such as reflexes along nerve tracts, humoral stimulants from active muscle, possible metabolic products of the muscle, and finally, the drainage of the blood from the central nervous system into the active musculature have been considered. The mechanism by which surgical stress leads to the release of growth hormone is also unknown (REICHLIN, 1966).

3. Interactions of Growth Hormone with Other Hormones

Since, unlike other pituitary hormones, growth hormone exerts its multiple effects not on a target organ, but on every cell in the organism, its action might be expected to depend on the metabolic equilibrium of each cell, which in turn is modified by other hormones. Indeed, practically all known hormones have been found to have at least some direct or indirect modifying effect on the production, release and/or metabolic action of growth hormone.

The interactions between growth hormone and catecholamines and between growth hormone and insulin have already been discussed (see p. 88). In addition to the interactions with insulin, it should be mentioned that glucagon has recently also been found to stimulate the secretion of growth hormone in certain conditions (MITCHELL, 1969; WEBER, 1970). Whether this is of any physiological importance remains to be seen.

a) Growth Hormone and Other Pituitary Hormones

ACTH is capable of stimulating the secretion of growth hormone in a similar way to insulin (ZAHND, 1970). This is apparently a direct effect of ACTH, which is not mediated through the glucocorticoids. *Vasopressin* and its synthetic analogue, lysine-vasopressin, are not merely potent stimulants of ACTH secretion (GWINUP, 1967), but also stimulate the release of growth

* For GRIH see p. 32.

hormone (GAGLIARDINO, 1967). However, this effect generally occurs only if vasopressin is administered in amounts far higher than those secreted physiologically, and it is questionable whether physiologically important interactions exist.

So far, no direct interactions have been demonstrated between TSH or gonadotropins and growth hormone.

b) Growth Hormone and Thyroid Hormone

Basal growth hormone levels in hypothyroid subjects are not usually significantly different from those found in normal subjects (IWATSUBO, 1967), but in hyperthyroidism the growth hormone levels tend to be raised (VINIK, 1968). In contrast, hypothyroid patients have a blunted response to stimuli of the growth hormone secretion such as insulin (IWATSUBO, 1967) or arginine (KATZ, 1969) administration. The awareness of this interaction is clinically important, because in untreated primary hypothyroidism the results of growth hormone secretion tests may be falsely pathologic and may lead to the erroneous diagnosis of growth hormone deficiency.

c) Growth Hormone and Glucocorticoids

On the basis of experience with children requiring treatment with large doses of glucocorticoids (e.g. for bronchial asthma) whose growth became stunted, pediatricians have long suspected that glucocorticoids might inhibit the secretion of growth hormone. It is now certain that the effects of glucocorticoids on growth hormone are manifold. Although there are still many inconsistencies in the experimental evidence, it may be stated that glucocorticoids do not impair the production of growth hormone in the pituitary gland itself, but they may inhibit its release into the bloodstream (FRANTZ, 1964). In addition, glucocorticoids seem to inhibit the peripheral growth-promoting actions of growth hormone, since glucocorticoids slow the growth rate even in hypopituitary dwarfs during substitution therapy with human growth hormone (SOYKA, 1965).

d) Growth Hormone and Sex Hormones

Although *testosterone* does not influence basal growth hormone levels, it increases the maximum growth hormone-secretory capacity in response to stimuli (MARTIN, 1968; see also p. 89).

In contrast to testosterone, *estrogens* do increase the basal growth hormone levels. Adult women generally have higher fasting growth hormone levels than children, men, or postmenopausal women, and the levels in men and children can be increased by the administration of estrogens (UNGER, 1965; FRANTZ, 1965).

The influence of synthetic sexual steroids such as medroxyprogesterone and oral contraceptives on the secretion of growth hormone has already been mentioned (see p. 89).

E. Pituitary Insufficiency, Panhypopituitarism

1. Classification

Before the leading role of the hypophysis in regulating the endocrine organs was known, this illness was referred to as "pluriglandular insufficiency" (CLAUDE) or "multiple endocrine sclerosis" (FALTA). These diagnoses are only rarely justified now (see Chap. XVIII). Isolated failure of each adenotropic hormone may occur (see p. 97f.), but failure of the whole anterior lobe is much more frequent and is described as panhypopituitarism or SIMMOND's disease. The most common causes are postpartum necrosis (Sheehan's syndrome) and destruction of the anterior lobe due to a pituitary tumor usually involving the posterior lobe as well. Failure of the posterior lobe does not, however, alter the clinical picture substantially. Diabetes insipidus is due to failure of the hypothalamic nuclei.

The clinical picture of hypopituitarism in its purest form is seen after therapeutic hypophysectomy for metastasizing carcinoma of the breast. The progression of symptoms can be followed in every detail in these cases. See p. 97f. for partial pituitary insufficiency.

2. Occurrence and Incidence

Panhypopituitarism is uncommon. SHEEHAN (1968) estimates an incidence of 100 cases of the most common variation of the disease, postpartum pituitary necrosis, in 1 million women. He found hypopituitarism in 8% of patients with mild blood loss during the delivery and in 53% of patients with severe hemorrhage. While SHEEHAN expected the development of pituitary insufficiency in over half all patients with severe postpartum hemorrhage, SCHNEEBERG (1960) found pituitary failure in only 4 of 35 cases with postpartum shock, and a follow-up of 235 patients in Zurich who lost more than 1 liter of blood at delivery did not reveal a single case of Sheehan's syndrome (WIESENDANGER, 1959). The incidence is certainly low,

and it is dependent on the population under investigation.

Panhypopituitarism is found more frequently in the female (65%), and is most common in the 3rd and 4th decades of life.

The relative incidence of the processes leading to pituitary failure is seen from the review by SHEEHAN and SUMMERS. Partial pituitary insufficiency is more frequent than was previously estimated and occurs more frequently than panhypopituitarism in chromophobe pituitary adenomas.

3. Etiology and Pathogenesis

Hypopituitarism is due to hypophyseal failure which may be caused by destruction of the hypophysis itself, or its connections with the hypothalamus, or by lesions in the hypothalamus. In most cases, four-fifths of those in the table given by SHEEHAN and SUMMERS, the defect is in the hypophysis itself. As a rule, one quarter of the original parenchyma of the anterior lobe can maintain the necessary adenotropic function of the hypophysis. Therefore, only 1–2% of intact tissue is usually found in severe cases. Weeks or years may elapse between the onset of the necrosis and the clinical appearance of hypopituitarism. The patients survive for 10–15 years on average, and sometimes even for over 40 years. Hypopituitarism becomes manifest in one fifth of the patients due to processes which interrupt the connections between the brain and the hypophysis. These are usually extrasellar cysts and tumors. As indicated in the discussion of the morbid anatomy of the hypophysis below, the etiology and pathogenesis of the basic diseases leading to pituitary failure are very varied. Necrosis of the adenohypophysis can be produced experimentally in rats with intravenous hexadimethrin bromide (KOVACS, 1967).

4. Pathological Anatomy

Hypopituitarism can be caused by damaged cerebral centers. The most frequent cause of panhypopituitarism however are necrosis of the hypophysis and its residual state (KERKHOVEN, 1966). These causes are followed in frequency by tumors and cysts. In contrast, inflammatory processes are relatively seldom responsible.

Symptoms due to partial failure, however, are more frequently produced by tumors and inflammatory processes of the hypophysis than by necrosis and sequelae.

Necroses of the anterior pituitary lobe are not infrequently found during the systematic patho-anatomical examination of postmortem cases. PLAUT (1952) demonstrated this during routine examination in almost 10% of male cadavers chosen at random (13 cases among 149 sections). SHEEHAN and STANFIELD (1961) found the same in 3% of their postmortem cases. Many necroses, however, must be considered as terminal complications.

The postpartum type of pituitary necrosis due to shock is most common. Vascular spasm seems to have a decisive pathogenic effect. Vascular thrombi, which can often be demonstrated in postpartum necrosis, are only a secondary phenomenon. In addition to the shock, a further factor is still needed to cause the necroses. Hypertrophy of the anterior lobe during pregnancy in the presence of a limited blood supply, the impossibility of expansion within the sella, the increase in the hypophyseal metabolism and postpartum involution with diminished blood supply are together responsible for the necroses. Apart from necroses during pregnancy, hypophyseal necroses have also been observed after shock resulting from hemorrhage, burns, epidemic hemorrhagic fever, sickle-cell crisis, insulin-shock treatment, and pulmonary embolism. These necroses, however, are usually less extensive than the postpartum type. Necroses of the anterior pituitary also occur in diabetes and hemochromatosis.

In addition, necroses of the anterior lobe occur frequently as a concomitant symptom in *primary and metastatic tumors* of the hypophysis. As a rule, these necroses are superficial. Infarcts, however, may also be due to tumor emboli. Like tumors, inflammation, particularly granulomatous processes, can also lead to accompanying necrosis.

Surgery and massive irradiation, intensive X-ray or proton irradiation, or implantation of radioactive yttrium or gold can cause hypophyseal necroses and lead to extensive scarring as a delayed sequela. Section of the stalk results in extensive necrosis because the portal vessels are a type of "end vessel". However, about 10% of the parenchyma is still retained since these areas are supplied by other vessels.

Frequently only scars are detectable and their cause can no longer be found. If the scars are very extensive, then the "empty sella" syndrome, a phenomenon seen quite often in autopsy, results. Cystic scars are denominated as cystic degeneration. Such cysts have no epithelial layers, in contrast to proper cysts. Interstitial fibrosis generally does not lead to hypopituitarism. The empty sella, however, may also result from increased pressure of the cerebrospinal fluid in the subarachnoidal space

of the gland, flattening but not destroying the pituitary. The empty sella syndrome may have different pathogenic mechanisms. It has been suggested that the incompetent diaphragma sellae may be an anatomical variant or may be caused by repeated swelling of the stalk in multiparous women (BRISMAN, 1972). The sella may be of normal size and shape, be balloon-like, deformed, or enlarged. For clinical features see p. 103 f.

Inflammation can lead to extensive destruction of the hypophysis, and therefore cause pituitary failure. *Purulent inflammation* usually arises from inflammatory processes in the surroundings. Abscesses of the anterior lobe develop almost without exception, spreading from sphenoidal sinusitis or possibly from a necrotic chromophobe adenoma. Abscesses developing after septic infarction have been described (SHEEHAN, 1965). Meningitis, osteomyelitis of the sphenoid bone and thrombophlebitis of the cavernous sinus can all spread to the hypophysis. Finally, inflammatory vascular processes also lead to hypophyseal destruction. *Diffuse, non-purulent, chronic inflammation* reminiscent of an autoimmune process, are worth mentioning. Both GOUDIE (1962) and HUME (1967) have seen hypophysitis of this type in a female patient with Hashimoto's thyroiditis. EGLOFF (1969) has observed an isolated lympho-plasmocytic hypophysitis in a patient with panhypopituitarism.

In addition to these more diffuse processes, granulomatous inflammations also have a pronounced effect. The tuberculoid giant cell granuloma is characteristic, usually involving only the hypophysis. The cause of the inflammation is unknown. Pituitary insufficiency is a late development since the granulomas can destroy the anterior lobe to a great extent. Women around the age of 55 are most often affected. Occasionally, tuberculosis, syphilis, sarcoidosis and certain mycoses also produce granulomatous inflammations with parenchymatous destruction.

5. Symptoms and Clinical Course

a) History, Psychological Changes

The illness, due to pituitary tumors and especially to postpartum necrosis, usually develops slowly over the course of years although on rare occasions it may present itself within a few weeks due to massive postpartum necrosis. The first symptoms arise 3 weeks after total removal of the hypophysis and generally become definitely manifest after 5 weeks. As a rule, growth hormone probably fails first, followed by the gonadotropins. Thy thyrotropic hormone then fails, and the adrenocorticotropic hormone is the last to disappear. There is minimal residual thyroid function and a basal adrenocortical secretion consisting entirely of aldosterone. Complete failure of the hypophysis is therefore compatible with life, even though life is severely impaired.

A change in menstruation is the first symptom in the female. The bleeding becomes scanty, short, and irregular, and finally fails to occur. Irregular bleeding can, however, occur. Occasionally menstruation starts again after cortisone substitution. Climacteric symptoms are usually absent but may occur (PURNELL, 1964). They may arise after delivery in postpartum hypophyseal necrosis and then gradually disappear. Failure of lactation and a hypoglycemic tendency are considered early symptoms. Pubic hair, shaved before delivery, does not return. Sensitivity to cold becomes apparent later, and the patients are usually always cold and dress warmly even in summer. Increasing tiredness to the point of adynamia finally causes the patients to become bedridden. Their speech is slow, monotonous, and hesitant, and later becomes slurred. Giddiness, tendency to faint, obstinate constipation, oliguria, and somnolence are further common symptoms. Typical symptoms are the hypoglycemic tendency and the inability to remain without food for even a short period of time. Impotency and loss of libido occur in the male. In general, the patients consult their physicians very late, since they are indifferent to their illness. Temporary diabetes insipidus may occasionally occur and has been described in about two dozen cases. Quite often it is primarily due to an organic lesion and later to a conditioned polydipsia (AGUILÓ, 1969), rather than to posterior pituitary necrosis and lack of vasopressin (EVANS, 1960).

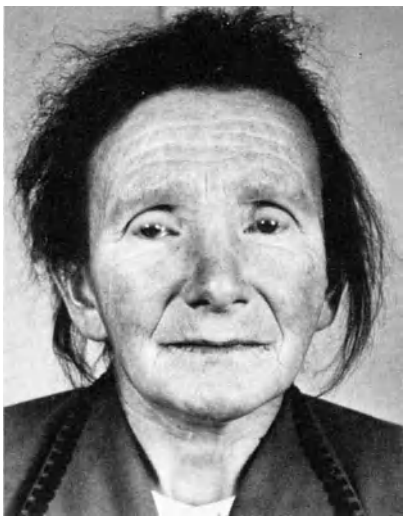
Failure of the adenohypophysis leads to severe psychological changes which may progress to psychoses. At the beginning, there is a lack of initiative, lack of interest, generalized retardation and apathetic depressive moods, making up the endocrine psychosyndrome. All motivation disappears completely in time. The sexual and motor impulses are lost first. Indifference develops into complete neglect of the surroundings as well as of the person. The patients decline intellectually, the amnestic psychosyndrome develops and they finally lead a no more than vegetative existence. States of the actue exogenous reaction type may be associated with the complete disappearance of psychological functions. These may progress to predominantly paranoid hallucinations, delirium, and finally to coma, usually associated with acute adrenal

insufficiency (BLEULER, 1964; KIND, 1958; LINDQVIST, 1966).

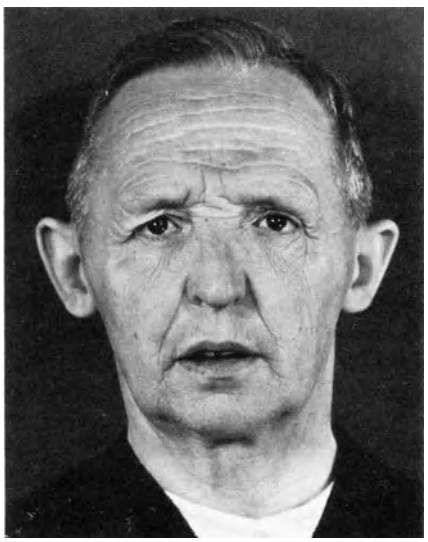
It is still not known whether substitution therapy, which compensates the somatic failure, can also reverse the psychological disorders completely or only partly improve them.

b) General Examination

The face of the patient with pituitary insufficiency is expressionless, as during sleep. The features are strangely blurred (Fig. 2). The patients appear uninterested, lifeless, and tired.



a



b

Fig. 2a and b. Facial expression in panhypopituitarism. a) 50-year-old female patient with SHEEHAN's syndrome (Prof. SCHÜPBACH, Inselspital, Bern). b) 61-year-old male patient with panhypopituitarism and diabetes insipidus, unknown etiology (TB?)

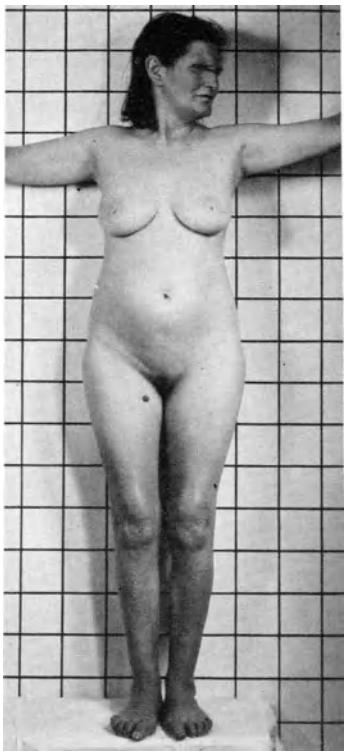


Fig. 3. 52-year-old female patient with Sheehan's syndrome. Complete absence of pubic and axillary hair. Striking pigment deficiency, especially in the areolas (Prof. SCHINZ, Radiological Dept. of the University of Zurich)

The skin can, but need not, show a myxedematous tendency. It is dry, but thin, delicate and "alabaster-like". The hair is dull, bristly, but not prematurely gray. The eyebrows may be absent or may be particularly scanty in the lateral third.

The fallow pallor of the thin, marble-like, translucent skin is striking (Fig. 3). Pigment deficiency is most noticeable in the normally strongly pigmented zones, such as the mamilla, the perigenital and the perianal areas. The lack of pigment is due to diminished melanin formation resulting from the failure of the hypophyseal melanocyte-stimulating hormone (see p. 294ff.). Measurable darkening of the skin can be achieved with MSH in patients with hypophyseal insufficiency. The inability to form melanin is demonstrated by the failure to turn brown after ultraviolet radiation. The cutaneous circulation is reduced; patients with hypopituitarism never blush.

Sweating and sebaceous secretion are also reduced or arrested.

The third characteristic is the loss of body hair (Figs. 3, 4). Pubic and axillary hair are totally absent in severe cases. The axillary hair can disappear within 2 months, whereas the

spontaneous loss of pubic hair takes 1–2 years. In men, growth of the beard and the moustache is scanty and there is no body hair. The diagnosis can be made with a high degree of certainty from these three visible findings; the expressionless face, neither young nor old; the striking pallor; and baldness of the body. Calcifications in the auricle, as in Addison's disease, have been observed (RANDALL, 1963).

The body weight is normal in 3/4 of the cases. SHEEHAN has conclusively corrected the long-established view, based on SIMMOND'S first description, that pituitary failure leads to cachexia (see Table 2).

Table 2. Weight in 103 cases of Simmond's disease. (From SHEEHAN, 1948)

	Fat	Higher than normal	Normal	Medium	Thin	Cachectic
At time of death	9	11	36	19	14	14
6 months before death	9	11	57	11	9	6

Emaciation may occur terminally although there is sometimes a weight gain at the beginning of the disease. Motor inactivity and reduced basal metabolic rate with unchanged appetite are probably the cause of this.

Subnormal temperatures may occur. The fact that patients with Sheehan's syndrome often have a smaller sella (<80 mm^2 in the lateral X-ray) than a control group (MEADOR, 1966) is worth investigating. Involutional processes and greater susceptibility of the smaller hypophysis have been considered.

Circulatory organs: Bradycardia is less pronounced than in myxedema. The hypotension is less severe than in Addison's disease. There is orthostatic hypotension. The heart is smaller than in Addison's disease. The small kidneys and the small liver are due to the splanchnomicria.

Ovarian failure leads to atrophy of the genital organs, and the vaginal smear shows no signs of estrogenic activity. Bleeding, however, may sometimes occur.

In the male, there is a secondary hypogonadotropic hypogonadism with tubular insufficiency at first, followed by interstitial failure (see p. 469 f.).

c) Laboratory Investigation

The diagnosis of a suspected case of hypopituitarism can be verified by demonstrating secondary thyroid, adrenal, and gonadal insufficiency and growth hormone deficiency.

See p. 160 for secondary hypothyroidism and its diagnosis (p. 241 f.).

BMR, radioiodine test, the protein-bound iodine and thyroxine determination can detect the hypothyroidism. The TSH test differentiates between primary and secondary hypothyroidism. Reduced TSH-reserve is demonstrated by the carbimazole test (see p. 242) or more recently, by the TRH-test (see Chap. VI, p. 242).

See p. 325 for secondary adrenal insufficiency. It can be differentiated from primary adrenal insufficiency by the 3-day intravenous ACTH test. Extremely low or absent urinary steroids (0–1 mg 17-keto and 17-hydroxysteroids) are suggestive of a secondary insufficiency. The aldosterone excretion is normal or within the lower limits of normal. Aldosterone secretion does not always rise sufficiently in response to salt deficiency (ROSS, 1960). Demonstration of an increased or decreased gonadotropin excretion is a good way of distinguishing primary from secondary hypogonadism (testes, p. 469 f.; ovaries, p. 585 ff.). Estimation of gonadotropin is of great diagnostic value especially in women after the menopause.

Impaired diuresis in hypopituitarism is partly due to the adrenal insufficiency. On the other hand, failure of growth hormone also causes a reduction in glomerular filtration and renal plasma flow. The defect can only be partially corrected by substitution with cortisone and thyroxine (FALKHEDEN, 1963). A pronounced hyponatremia of 110–120 mEq/l is not uncommonly found in untreated cases of pituitary insufficiency, especially in response to stress such as surgery. Strangely, this hyponatremia is well tolerated, and regresses with cortisone alone. The pathogenesis is not completely clear (BETHUNE, 1965; GASTINEAU, 1967). Renal sodium loss is not usually present, and the serum urea, in contrast to the dehydration in ADDISON'S disease, remains low. Excessive water retention has been suspected to be due to inadequate vasopressin secretion (AUBRY, 1965) since lack of cortisone causes a fall in the osmotic stimulus threshold. The fact that water deprivation does not increase the sodium concentration and that it is possible to correct the disorder with cortisone alone makes the overproduction of vasopressin appear unlikely. The extracellular space is enlarged. Water overloading, which

was previously recommended as a diagnostic test, is dangerous, involving the risk of water intoxication with brain edema and epileptic fits. Unexplained hyponatremia should suggest pituitary insufficiency and is more pronounced in hypopituitarism due also to the failure of growth hormone. The insulin tolerance test best demonstrates the deficient counter-regulation. The blood sugar falls rapidly to low values and does not rise promptly or rises only inadequately. The typical rise in the level of growth hormone is absent. This test, however, also involves a certain amount of risk and should be used cautiously. Glucose for intravenous injection must always be ready to terminate the test.

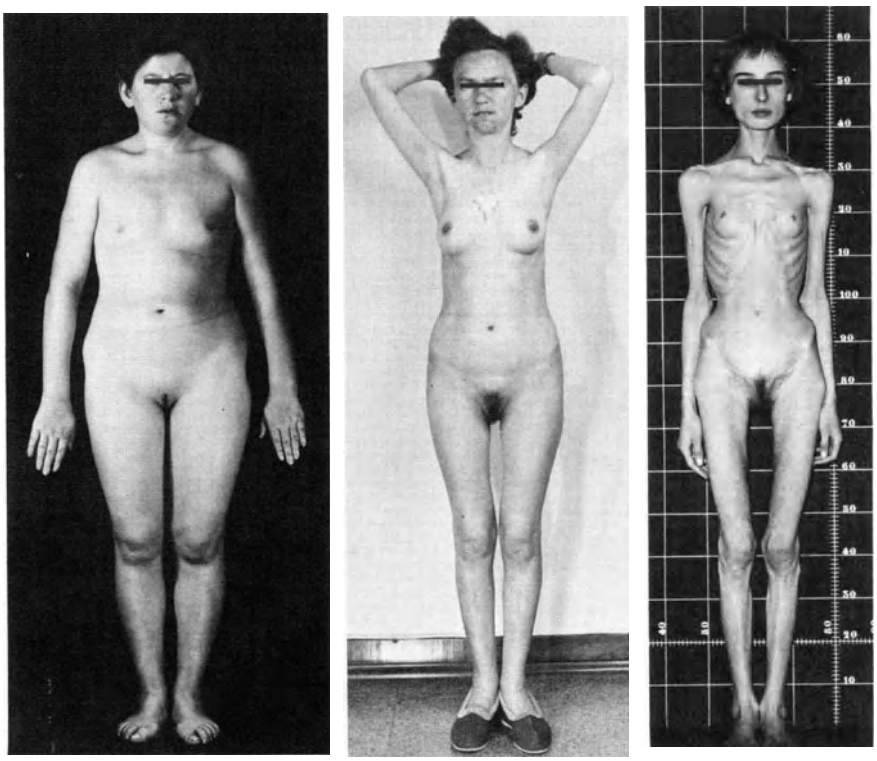
Blood findings: the erythrocyte sedimentation is normal. There is mild anemia, at first hypochromic and later hyperchromic, due to failure of the adrenal glucocorticoids and a pituitary factor. The white blood cell smear shows an increase in eosinophils and lymphocytes.

Achylia, which may prove to be refractory to histamine, has sometimes been observed.

6. Differential Diagnosis

Panhypopituitarism must first be differentiated from post partum endometrial amenorrhea, occuring very seldom. In the latter condition there are no clinical signs of failure of the thyroid and adrenals. Hormone estimations are normal. The differentiation between pronounced anorexia nervosa and hypopituitarism is not difficult. The question of pituitary function in anorexia often comes into consideration because of the wrong dogma which has existed for years that pituitary insufficiency leads to cachexia. Anorexia does, however, always have some features in common with pituitary insufficiency; the basal metabolic rate is always reduced, and there is amenorrhea in both conditions.

In anorexia, however, there is a hypometabolism without hypothyroidism. Steroid excretion is diminished in anorexia, but never to the same extent as in hypopituitarism. The adrenals are not found to be atrophic, and the adrenal insufficiency is only relative. The amenorrhea has the same cause in both conditions, and the gonadotropins are usually not demonstrable in anorexia (see p. 591). In anorexia, FSH production ceases under central nervous system influences. Emaciation is suggestive of anorexia, and makes hypopituitarism unlikely (Fig. 4). In typical cases of anorexia the skin is covered with lanugo hair. Pubic and axillary hair may be scanty but is never completely



a b c

Fig. 4a–c. Differential diagnosis of hypopituitarism. a) 26-year-old female patient with hypopituitarism, due to a chromophobe adenoma. b) 35-year-old female patient with the sprue syndrome. Chloasma-like pigmentation of the forehead and chin, axillary hair absent, pubic hair scanty. c) 19-year-old female with anorexia nervosa (KspZ)

absent as it is in pituitary insufficiency. The psychological changes in the two diseases are very different. The anorexic patient is shy and uncommunicative, but is not apathetic, and can be very lively and enterprising. The physical deterioration leads only terminally to apathy. Differentiation can be difficult in the terminal stages of both illnesses. The insulin tolerance test with growth hormone determinations makes differentiation possible. This test is, however, not without risk. In anorexia, the blood sugar will return to normal in response to hypoglycemia, whereas in hypopituitarism, the blood sugar usually remains low until glucose is given.

The malabsorption syndrome may simulate hypopituitarism. Scanty pubic and axillary hair, adynamia, anemia and low ketosteroid excretion are common to both conditions. However, the waxy pallor of hypopituitarism is absent in sprue (Fig. 4b), and in contrast, there is quite often pigmentation of the skin.

Differentiation from primary myxedema can be difficult when the secondary hypothyroidism dominates the clinical picture. The TSH test, the TRH test and the demonstration of the failure of other endocrine systems establishe the diagnosis.

Primary adrenal insufficiency is distinguishable mainly by the pigmentation. "White" Addisonians can be difficult to differentiate from hypopituitarism since the basal metabolic rate can also be reduced in Addison's disease, and gonadal dysfunction may also occur. The ACTH-test repeated over 3 days can differentiate the two diseases. Clinical distinction between hypopituitarism and Schmidt's syndrome (primary hypothyroidism and adrenal cortical insufficiency (see Chap. XVIII) can be difficult. This syndrome is probably underlaid by autoimmunological factors and often occurs with diabetes mellitus. The presence of gonadotropin, primary hypothyroidism in the TSH-test, primary adrenal insufficiency indicated by the ACTH-test, and serum antibodies to the thyroid and adrenal tissues suggest Schmidt's syndrome.

Hypoglycemic states can involve islet-cell adenoma in the differential diagnosis. Evidence of thyroid, gonadal, and adrenal insufficiency proves the pituitary cause.

Finally, in the male, a differential diagnosis between primary or secondary hypogonadism and hypopituitarism has to be made. The differentiation is based on the estimation of the gonadotropins, the testicular biopsy and the demonstration of hypothyroidism and adrenal insufficiency.

7. Special Forms

a) Partial Hypopituitarism

The diagnosis of partial hypopituitarism has been facilitated by the development of diagnostic tests. It occurs more commonly than panhypopituitarism in chromophobe adenomas and after therapeutic hypophysectomy. The effects are dependent upon the extent of the destruction and the duration of the illness. They can also arise after failure of the hypothalamic regulation centers. Partial generalized insufficiency can occur which is only detectable by the different reserve tests, or there may be a selective failure of every single adenotropic hormone and of the growth hormone. Not infrequently, partial hypopituitarism is present at the beginning and may progress to panhypopituitarism over the course of months or years. Hypogonadotropic hypogonadism was considered to be the most frequent partial hypopituitarism (see p. 469), isolated secondary hypothyroidism to be less common (see p. 160), and isolated secondary adrenal insufficiency even rarer (see p. 325). The last condition may also arise after successful removal of an adreno-

Table 3. Differential diagnosis between anorexia mentalis and hypopituitarism. (After ESCAMILLA)

	Anorexia	Hypopituitarism
Symptoms common to both diseases:		
Secondary amenorrhea (FSH absent)	+	+
Asthenia	+	+ +
Hypotension	+	+ +
Hypometabolism (reduced BMR)	+	+ +
Symptoms occurring in one disease only:		
Loss of weight	+ + +	0
Pallor	0	+ + +
Loss of pubic and axillary hair	+	+ + +
Eosinophilia	0	+ +
Average age	21	41
Onset	After puberty	After pregnancy
Insulin tolerance	Normal	Reduced
ACTH-test	Normal	Normal, only after repeating 3 times

cortical adenoma. Recently, however, isolated failure of growth hormone without clinical symptoms appears to be the most common adult endocrine disorder secondary to chromophobe adenoma. This failure can only be demonstrated by the radioimmunological methods for estimating growth hormone in combination with the insulin tolerance test (see p. 123) (RABKIN, 1966). Selective failure can occur in all combinations (DAYER, 1959). Among 69 chromophobe adenomas there were 10 cases showing no failure, 16 cases with failure of all 3 adenotropic hormones, 22 cases of one isolated failure, (17 hypogonadism, 3 hypothyroidism, 2 adrenal insufficiency) and 21 cases of secondary failure of 2 glands (OBERDISSE, 1957). Secondary hypogonadism and secondary adrenal insufficiency appear to occur more frequently together than is attributable to chance and have been collectively termed "basophil failure" (MADDOCK, 1951).

Isolated failure of the growth hormone leads to pituitary dwarfism. For discussion of selective failure of TSH in pseudohypoparathyroidism the reader is referred to Chap. XIV.

Selective secondary hypothyroidism may cause pituitary impairment leading to adrenal cortical insufficiency. Thus it may simulate panhypopituitarism.

Hypopituitarism, due probably to a hypophysitis caused by autoimmunological factors, can occur with other immunological disorders in the organism (HUME, 1967) (see Chap. XVIII).

A strange form of partial hypopituitarism with giantism and normal or increased growth hormone effects has been described by GOLDMAN, 1963, and SARVER, 1964.

b) Pituitary Dwarfism

A. PRADER and M. ZACHMANN

Pituitary dwarfism is due to an inhibition of growth associated with a deficiency of somatotropic hormones. It begins during infancy. Since differentiation between a hypothalamic disorder and pituitary insufficiency is impossible in some patients, it would be more correct to speak of "hypothalamo-pituitary" dwarfism. Unfortunately, the term "pituitary dwarfsm" is often used rather uncritically for all cases of small stature. It must be stressed that pituitary dwarfism should only be diagnosed when growth hormone deficiency is demonstrable. Patients affected by this deficiency are uncommon in comparison to the frequency of small stature.

Thyroid, adrenocorticotropic and gonadotropic functions are disturbed as well as growth in classic cases. There are, however, patients with isolated growth hormone deficiency and intact function of all other anterior pituitary hormones. Such cases are probably not very rare, but they can only be detected by refined diagnostic methods. Apart from this, there are also known forms of growth hormone deficiency combined with TSH, ACTH or gonadotropin deficiency. Thus, pituitary dwarfism is possible throughout this spectrum, from isolated growth hormone deficiency to the complete picture of panhypopituitarism.

The following forms of pituitary dwarfism can be differentiated on the basis of clinical and etiological features:

1. Isolated growth hormone deficiency presents as dwarfism in the presence of intact function of all the other anterior pituitary hormones. The clinical picture is hardly different from that in other types of nonendocrine dwarfism (primordial constitutional dwarfism, Chap. XIX). The differentiation from other forms is, however, of importance because of the therapeutic possibilities in the case of a growth hormone deficiency. There are idiopathic (sporadic) and hereditary (recessive inheritance; RIMOIN, 1966) forms of isolated growth hormone deficiency. Since puberty, although delayed, arises spontaneously in these patients, they are also described as "sexual ateliotics" in older classifications (GILFORD).

2. Growth hormone deficiency with multiple anterior pituitary insufficiency can also occur idiopathically or can be inherited. Boys are affected by the idiopathic form more frequently than girls. (This also applies to the form described in 1., above.) This form of pituitary dwarfism may be due to cerebral trauma during birth or to asphyxia at birth in many cases. The possibility of an organic disorder must be considered particularly in cases with multiple failure; an intra- or suprasellar tumor, usually a craniopharyngioma, can cause this form of pituitary dwarfism (Fröhlich' dystrophia adiposogenitalis in the original meaning of the term). Other organic causes include malformations in the region of the pituitary or hypothalamus and finally, traumatic damage (fracture of the base of the skull with hypophyseal hemorrhage or direct traumatic influence on the pituitary). Patients suffering from multiple failure with gonadotropin deficiency were formerly also referred to as "asexual ateliotics" (GILFORD).

3. A secondary, symptomatic growth hormone deficiency occurs with severe general disease and also occasionally with malnutrition. Primary hypothyroidism can also lead to inhibition of growth hormone secretion, as can corticosteroids, which would at least partly explain the small stature in Cushing's syndrome

and following long-term corticosteroid therapy with high doses. A temporary growth hormone deficiency occasionally results in small stature in children brought up in unfavorable social conditions, particularly in children deprived of maternal affection (POWELL, 1967, "emotional deprivation", Chap. XIX).

4. The possibility of a functional deficiency of growth hormone in the presence of normal secretion (peripheral resistance or inactive growth hormone) has not been fully studied. Thus, for example, peripheral resistance to growth hormone is assumed to be the cause of the small stature of pygmies, in whom normal growth hormone concentrations were found in the plasma during stimulation tests. Apart from this, there also appear to be hereditary forms of dwarfism where high levels of GH are found (LARON, 1968) but where presumably a mediator, the sulfation factor or somatomedin (see p. 88), is deficient.

Growth in patients with pituitary dwarfism is usually normal in the first two years of life and then becomes progressively slower (Fig. 5). On rare occasions, the growth deficit appears in the first months of life. Body weight and length are usually normal at birth. When gonadotropins are absent, the pubertal growth spurt and postpubertal termination of growth fail to occur. The majority of patients with multiple failure continue to grow throughout life, although the increase after the 20th year is only a few cm. The adult height of untreated patients lies between 100 and 140 cm.

As a rule, there is mild obesity in spite of a definite lack of appetite in all forms of pituitary dwarfism. This is a reflection of the absence of the lipolytic action of growth hormone. The obesity involves the trunk in particular (Fig. 6). Body

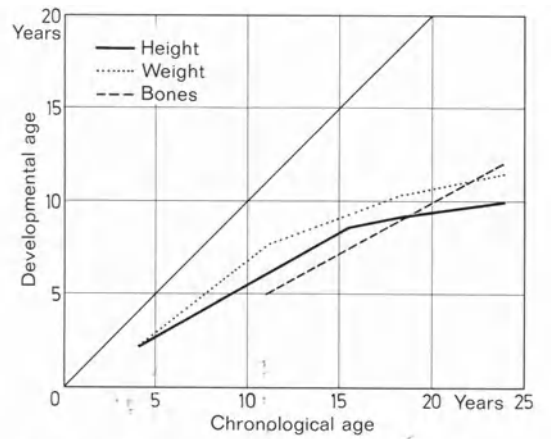


Fig. 5. Development of height, weight and bones from 5th to 24th year in a man with pituitary dwarfism. Note the general retardation of development and the continuous growth even after 20th year

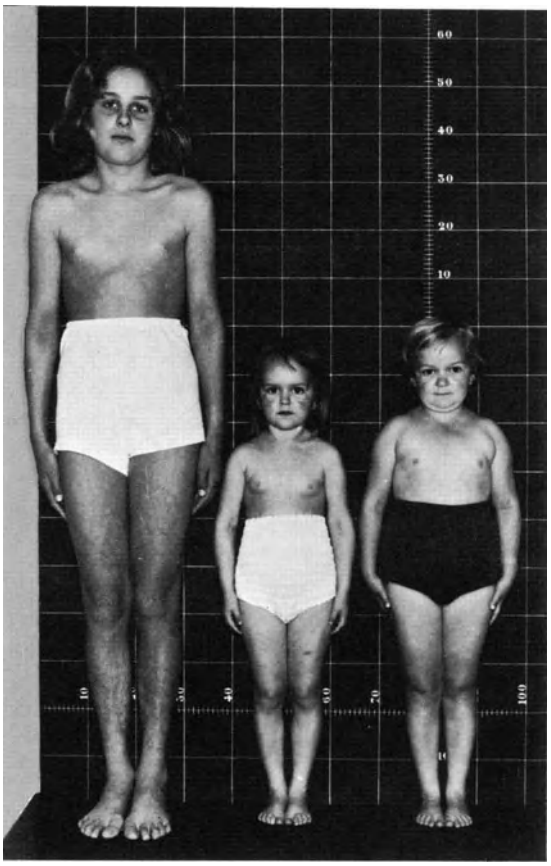


Fig. 6. Two girls with familial pituitary dwarfism (isolated growth hormone deficiency) and a girl of normal height of about the same age

	Age	Height age	Bone age
Healthy girl	14	14	–
Patient, center	13.9	3.7	8.3
Patient, right	13.4	4.1	10.5

proportions usually correspond to normal values, except that the head is relatively large. Despite the small stature, slightly eunuchoid proportions develop after the 10th–15th year when gonadotropin is also deficient. Hands and feet are often exceptionally small and doll-like (acromicria) and the face retains the round shape and the soft, doll-like features of an infant.

Bone development is retarded. The degree of retardation varies individually and is greater in the presence of a TSH deficiency. As a rule, bone development and increase in height are retarded to roughly the same extent. Development of the paranasal sinuses is also delayed. The *sella* is occasionally enlarged in cases with tumors. In other cases it is often smaller, but no diagnostic importance can be attributed to this finding.

Necrosis of the head of the femur, which is reminiscent of Perthes's disease, is another skeletal finding occasionally found in association with a simultaneous TSH deficiency (see p. 170). Development of the teeth is also definitely retarded, though usually to a lesser extent than the bone development.

The *skin* is tender, thin, and often rather dry. The turgor is sometimes slightly reduced and the extremities tend to be cool. During the third decade or later, the skin of untreated patients shows fine folds and wrinkles, especially the face, giving the patients a senile appearance in striking contrast to the infantile body stature.

Intelligence is commensurate with age, so that in contrast to hypothyroid dwarfs, these children can usually keep up quite well in school as long as their physical strength is adequate and hypoglycemic attacks do not arise. Psychological infantilism is often present, which is especially pronounced in cases where puberty fails to develop (see also gonadal dysgenesis, Chap. XII). The sense of shame is often extreme, as is the sensitivity of these patients to disparaging and insulting remarks. This indicates just how much patients suffer from their appearance and from the fact that they are taken to be younger than they actually are.

Except in cases with a suprasellar tumor, few pathologic findings can be detected in the *nervous system*. An uncharacteristic, slight abnormality is occasionally found in the electroencephalogram. In cases where tumors are present, endocrine symptoms may precede the neurological ones, which are often not apparent until school age or early adulthood since the craniopharyngioma grows slowly. These symptoms can appear at any time in the case of other tumors, which are, however, very uncommon. Signs of a tumor are: headache, vomiting, visual disturbances, loss of visual fields (mainly bitemporal hemianopsia), papilledema, bursting of cranial sutures and enlargement of the sella. Since craniopharyngiomas usually become calcified, a suprasellar calcium shadow on the X-ray is particularly typical (Fig. 7).

Metabolic findings are best classified by the individual anterior pituitary hormones. It must be mentioned that, as in acquired pituitary insufficiency in the adult, no parathyroid insufficiency has been observed.

Growth hormone deficiency causes few direct metabolic findings which can be recognized: phosphorus and alkaline phosphatase in the serum may be diminished and cholesterol may be elevated. Nitrogen excretion in the urine is often higher than in healthy subjects but can only be assessed if the patients keep to a consistent diet. Single estimations of growth hormone

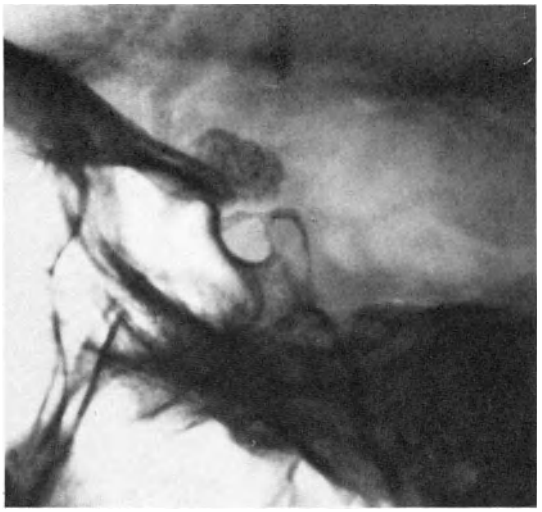


Fig. 7. Suprasellar calcification in craniopharyngioma

in the plasma are of little value since the concentration is subject to extremely wide physiological variations. Multiple estimations after stimulation of growth hormone secretion are therefore essential. Stimulation can be produced by insulin hypoglycemia, intravenous administration of arginine, a protein meal, or other stimuli. In the healthy subject the growth hormone concentration in the plasma then attains a maximum 30–60 minutes after insulin, and somewhat later with other methods of stimulation. Absolute values vary according to the laboratory. Fasting levels vary between 0 and 5 ng/ml plasma, and after maximum stimulation, the levels are between 10 and 20 ng/ml or higher. If the plasma concentration in a patient with dwarfism increases significantly or fasting values are very high a growth hormone deficiency can be excluded with certainty. If there is no rise, deficiency is possible but not yet confirmed. In doubtful cases, a nitrogen retention test can help. This test is based on the more pronounced metabolic action of growth hormone in patients with endogenous deficiency than in control cases with intact pituitary function. The long duration is a disadvantage associated with this test; patients receive a consistent diet chosen by themselves for 10 days. The first 5 days are used as a control period for estimation of the mean nitrogen excretion. Over the next 5 days, growth hormone is given intramuscularly every day in a dose of 2 mg/m^2 body surface area and the mean nitrogen excretion is calculated from the 2nd to the 5th day of the treatment period. The nitrogen retention as a percentage of the mean nitrogen excretion during the control period gives a good demarcation between patients with growth hormone deficiency and those without (PRADER, 1968).

Thyroid insufficiency is rarely manifest in the presence of *TSH deficiency*. The patients frequently give the impression of being alert and active and show no signs of the typical skin symptoms. Constipation may also be absent. Not uncommonly, the skin is found to be suspiciously dry and rough. Less frequently there are changes reminiscent of Perthes's disease in the head of the femur. PBI and radioiodine investigations give low values. Uptake of ^{131}I becomes normalized after 3 days' treatment with TSH, or sometimes a week of treatment is necessary. Blood pressure and basal metabolic rate (to be interpreted with reservation in children) are reduced, but are never as low as in classic primary hypothyroidism. The Achilles' tendon reflex time is prolonged. Serum cholesterol is increased, but this must not be interpreted as a sign of TSH deficiency only, since it is also elevated in isolated growth hormone deficiency. This is also applicable to phosphorus and alkaline phosphatase values, which are often reduced. See p. 123f. for the relation between growth and serum phosphorus.

ACTH deficiency seldom leads to manifest impairment of adrenocortical function although latent insufficiency can be demonstrated under increased stress. This is indicated by the case history in which there is occasionally a record of tendency to collapse and to become exhausted during great physical exertion and generalized illnesses. Individual patients suffer from hypoglycemic attacks, especially during childhood, as a result of the simultaneous failure of growth hormone and ACTH. The fasting blood sugar is slightly diminished or normal. More thorough investigation of carbohydrate metabolism yields findings similar to those in acquired pituitary insufficiency in the adult (p. 96); the rise in blood glucose after administration of insulin is definitely delayed. It may, however, be absolutely normal in single cases, even in the absence of growth hormone and ACTH. On the other hand, the rise in plasma cortisol after insulin administration is always inadequate in the presence of ACTH deficiency. If there is a history of hypoglycemic states, the insulin tolerance test must be carried out initially with only half the conventional insulin dose (2 U/m^2) because of the danger of hypoglycemic shock (Chap. XIII). In contrast to insulin, which causes liberation of ACTH by higher centers, high doses of the synthetic preparation, lysin-vasopressin, probably act on the pituitary, causing direct release of ACTH. A theoretical differentiation can thus be made between a hypothalamic and a pituitary lesion by comparing the cortisol increase after insulin and that after lysin-vasopressin. In fact, there is often no rise in plasma cortisol after insulin in cases with suprasellar tumors, and a normal increase after lysin-vasopressin. Experience with this test is, however, still limited. Delayed water excretion in the water tolerance test and the return to normal after administration of synthetic depot ACTH for 1–2 days are further characteristics of an ACTH deficiency. Electrolyte concentrations in the serum are usually normal, whereas the sodium is often slightly elevated in the sweat and the sodium/potassium quotient is raised. Excretion of 17-ketosteroids and 17-hydroxycorticosteroids is unaltered in the infant, but persists at the infantile level in later life. See Chap. VII, p. 385ff. for other examinations of the pituitary-adrenocortical system. In summary, ACTH deficiency in pituitary dwarfism leads to a latent adrenocortical insufficiency which is usually less severe than in Sheehan's syndrom. Development of the gonads and genitalia remains at an infantile stage in pituitary dwarfism with simultaneous *gonadotropin deficiency*. Secondary sexual characteristics fail to develop. Excretion of testosterone, 17-ketosteroids and gonadotropin correspond to that in the healthy infant. In other words, there is a severe hypogonadotropic hypogonadism (p. 469). The complete absence of sexual hair shows that not only gonadal function, but also the production of androgenic adrenocortical hormones is impaired (see adrenarche, Chap. XIX). Gonadotropin deficiency can, however, only be unmistakably recognized by clinical examination when the patients have reached the bone age at which puberty normally begins (boys approximately 13, girls approximately 11 years, Chap. XIX). Since bone development is generally significantly retarded in growth hormone deficiency, puberty is also often delayed in isolated growth hormone deficiency.

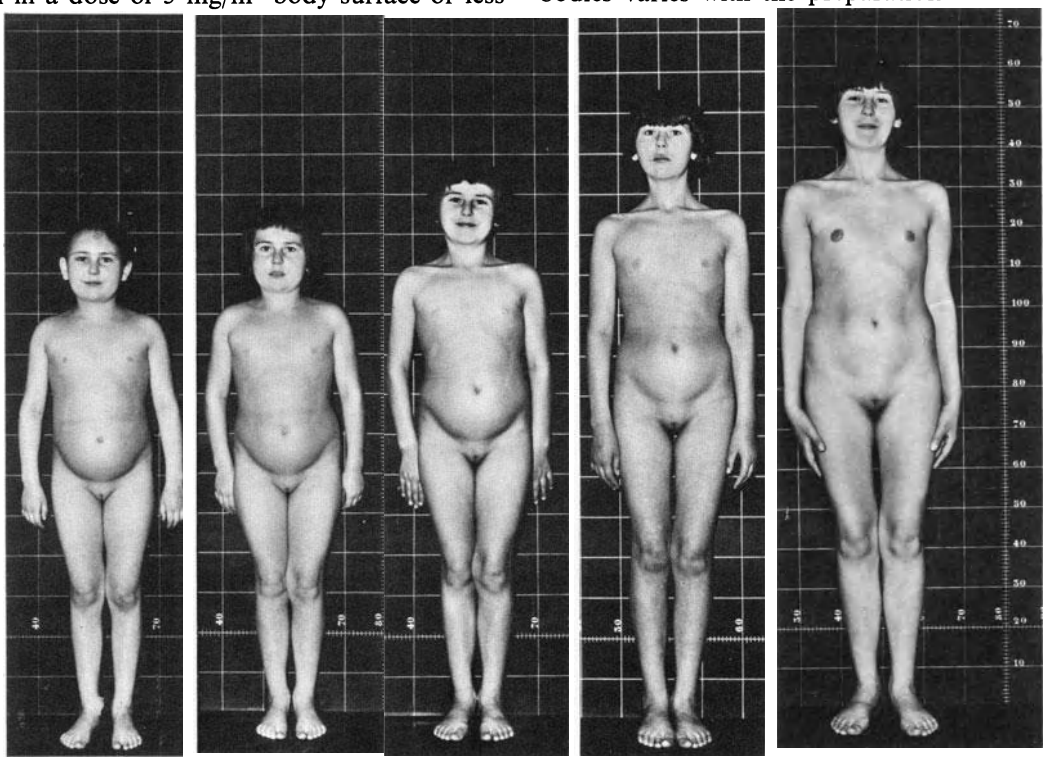
The *diagnosis* is easy in the adult. Practically no other diagnosis need be considered in the case of a dwarf with open epiphyses and no secondary sexual characteristics. The situation is much more difficult in the child, especially in the case of an isolated growth hormone deficiency; it is almost impossible to differentiate pituitary dwarfism from the much more common forms of dwarfism by clinical examination alone (Chap. XIX). The insulin tolerance test, with estimation of glucose, growth hormone and cortisol in the plasma, is particularly valuable in the assessment of this situation, since growth hormone deficiency and ACTH deficiency can be detected at the same time. Refer to Chap. XIX for a discussion of the differential diagnosis of dwarfism.

As far as duration of life is concerned, the *prognosis* is good. If treatment is not started

soon enough, the dwarfism, infantile appearance, and limited physical energy, impose severe restrictions on the patient's way of life. The physical capacity is usually astonishingly good, especially if there is no ACTH deficiency. The prognosis must be assessed with caution in cases with tumors, since recurrences or neurological complications are possible with craniopharyngiomas for example.

Apart from neurosurgical intervention when tumors are present (p. 122), continuous substitution therapy is the only possible form of treatment. The theoretically correct treatment consists of administration of pituitary growth hormone and the missing adenotropic hormones. For the moment, however, this can be realized only in some cases.

An impressive acceleration in growth rate can be achieved with *human growth hormone*, and normal adult height can be attained if treatment is started early enough (Fig. 8). Unfortunately, the hormone has not yet been synthesized, so that it is available only as extracts from human pituitary glands. Therapeutic possibilities are therefore limited to a few centers, and the amounts available are not sufficient to treat all patients with pituitary dwarfism. Two injections of an active preparation in a dose of 5 mg/m^2 body surface or less per week are sufficient to produce an optimal effect on growth (e.g. in the case of extracts prepared according to RABEN, WILHELMI or ROOS, 1 mg contains about 1.5 USP units). As a rule, a growth rate much faster than the normal rate is observed during the early months of treatment. Thus, not only is normal growth restored, but "catch-up" growth also occurs. Afterwards, the rate of growth slowly decelerates and assumes a normal level appropriate to the age (Fig. 9). Growth hormone promotes bone maturation in addition to growth. In contrast to anabolic steroids or testosterone, it accelerates bone maturation to a lesser extent than gain in height. Successful treatment also results in the reduction of subcutaneous fatty tissue. In spite of this, weight increases in the same way as height, an indication that muscle mass is increasing while adipose tissue is decreasing. Antibodies to growth hormone arise in a certain percentage of treated patients. In many cases they are unimportant, but they can arrest growth, especially when high titers can be demonstrated. Little is known about the cause of the formation of antibodies. It is probable that a certain denaturation of the growth hormone during extraction has some effect, since the frequency of the occurrence of antibodies varies with the preparation in use. The



$10^{11}/_{12}$ $12^{8}/_{12}$ $14^{7}/_{12}$ $17^{0}/_{12}$ $19^{0}/_{12}$

Fig. 8. Action of human growth hormone in a girl with pituitary dwarfism (panhypopituitarism following surgical treatment of a craniopharyngioma at the age of 10 years 11 months). Pigmentation of the mamilla at the age of 19 is due to the additional treatment with estrogens

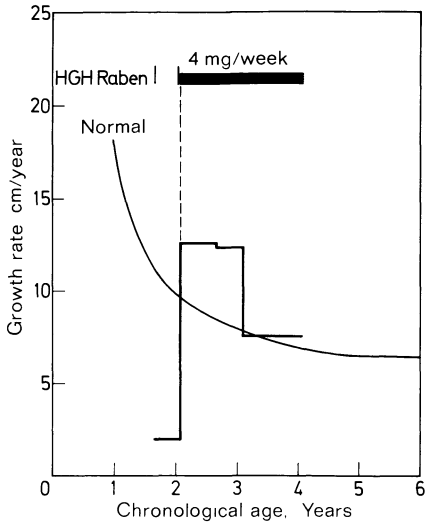


Fig. 9. Action of human growth hormone on the growth rate in a boy with pituitary dwarfism (idiopathic isolated growth hormone deficiency). At first the rate of growth is greater than normal ("catch-up" growth) and then it becomes normal for the age

tendency to form antibodies, however, also appears to have genetic reasons. *Attempts at promoting growth with anabolic steroids and testosterone* are only justified when human growth hormone is unavailable. These preparations often have a temporary favorable effect. Since they frequently promote bone maturation to a greater extent than growth, they have a less favorable effect than human growth hormone on the future adult height. Their use in substitution therapy for gonadotropin deficiency will be discussed. The question of using TSH for *substitution in TSH deficiency* does not arise, since the preparations available eventually become inactive. It is therefore necessary to institute treatment with thyroid extracts (preferably with Na-L thyroxine or a mixed preparation of L-thyroxine and triiodothyronine). The dose necessary varies widely for individual patients. A small daily dose of 50–100 µg is usually given at first, and is adjusted according to the serum cholesterol and the reflexogram of the Achilles tendon. Growth hormone can only be fully effective in a euthyroid state.

Substitution for ACTH deficiency is absolutely essential when hypoglycemic episodes occur. Regular intramuscular administration of synthetic depot ACTH can be considered (e.g. 0.05 mg Synacthen Depot every 2–3 days). Oral administration of hydrocortisone (e.g. 6.25 to 12.5 mg twice daily) or prednisone (1.25–2.5 mg twice daily) is simpler. Since glucocorticoids have a growth-inhibiting action, the dose should be kept as low as possible (ACTH is perhaps better in this respect). When correct indications and dosages are applied the physical state can be successfully improved and hypoglycemic attacks prevented without causing CUSHING'S symptoms or impairing growth. Bearing in mind the remarks above about treatment with anabolic steroids and testosterone, *substitution with sexual steroids* should be postponed as long as possible in hypogonadism, until a body height appropriate to the onset of puberty has been achieved with growth hormone therapy. In practice, however, commencement of the treatment must be adjusted to the psychological state. Treatment with gonadotropin would theoretically be preferable in these cases too, since this is the only therapy which permits maturation of the gonads. Such a treatment is, however, complicated and expensive. It is simpler to start with sexual steroids which bring about secondary sexual characteristics and when possible also a pubertal growth spurt. Later, when growth ceases, administration of human gonadotropins can be considered to produce gonadal maturation and possibly fertility. Refer to Chap. XII for gonadal dysgenesis, the selection and dosages of sexual steroids and gonadotropin (p. 599ff.) the ovaries, and (p. 475) the testes, and p. 475ff. for hypogonadotropic hypogonadism. It is interesting to note that the androgenic and growth-promoting action of testosterone is often much reduced in the presence of a simultaneous growth hormone deficiency compared with that in other patients.

The recent determination of the amino-acid sequences of some hypothalamic releasing hormones and the possibility of preparing some of these compounds synthetically opens new aspects with respect to the treatment of patients with "hypothalamic" rather than "pituitary" dwarfism.

So far, synthetic TRH and, to some extent, LHRH are available for research. It is conceivable that in the future it will be possible to treat some "hypothalamic-pituitary" dwarfs with the appropriate releasing hormones, notably with GHRH, rather than with human growth hormone and the other hormones mentioned above. GHRH will probably be easier to synthesize than human growth hormone, which is a much larger molecule. In this way, a larger number of patients could benefit from successful treatment, while the limited quantities of human growth hormone could be reserved for the patients with true "pituitary" dwarfism.

c) The "Empty Sella Syndrome" (KAUFMAN, 1968; CAPLAN, 1969; BRISMAN, 1972)

The pituitary function in this uncommon syndrome (see also p. 92) may be entirely normal

or there may be a partial pituitary insufficiency, usually not clinically manifest, affecting especially growth hormone and luteinizing hormone in stimulation tests. The pituitary is flattened and located in the lower posterior part of the sella. The sella may vary from normal radiological or anatomical appearance to globular, balloon-shaped forms with thinning of the posterior processus clinoidei. In about half the cases the main symptom is recurrent rhinorrhea and occasionally visual symptoms have been observed, resulting from dislocation, stretching or kinking of the optic nerves (OLSON, 1972). The syndrome can be detected by pneumoencephalography and is more common in females.

d) Hypopituitarism due to Hypothalamic Disorders

α) Dystopia of the Posterior Pituitary

Undisturbed contact with the neurohypophysis is essential for perfect development and function of the adenohypophysis. This is seen in dystopia of the posterior pituitary, a rare syndrome with pituitary insufficiency and various malformations (PRIESEL, 1927). Three different forms of dystopia of the posterior lobe of the pituitary are possible when the migration of the posterior lobe to the sella is prematurely arrested. In the tuberal form, the posterior lobe lies at the tuber cinereum, in the infundibular form, it is in the hypophyseal stalk, and in the opercular form it is on the anterior lobe. Opercular and infundibular dystopia do not necessarily produce symptoms. The adenohypophysis may remain small in the tuberal form, and usually a partial hypopituitarism develops, with stunted stature, hypogonadism, partial adrenal insufficiency, and various malformations, especially of the urogenital tract and of the heart. Secretion or regulation of STH, the gonadotropins, and to some extent of ACTH is probably impaired. Thyrotropin appears to be released normally. A striking frontotemporal baldness affecting women as well as men, cataracts and creatinuria are suggestive of a relationship to STEINERT's myotonic dystrophia. The syndrome can only be suspected on the basis of clinical examination. Dystopia of the posterior lobe is to be proved by necropsy.

β) Dysplasia of the Sella Turcica

In general, a small or normal sella turcica is of no significance, whereas an abnormally flat sella seems to indicate hypophyseal malformation (LUNDBERG, 1966). Hypogonadism, dwarfism, impaired ACTH regulation, cataract, and frontotemporal baldness can occur in varying degrees of severity and in different combinations.

γ) Chronic Inflammatory Processes

such as sarcoidosis, tuberculosis, the Schüller-Christian disease, and further metastases can lead to selective failure of the adenotropic pituitary hormones and particularly to hypogonadism (see p. 469f.). For hypopituitarism combined with diabetes insipidus in hypothalamic processes, the reader is referred to p. 93.

e) "Functional Hypopituitarism"

Different authors have assumed that the adenohypophysis limits its function as regulator of the metabolic processes in undernourishment and starvation, resulting in functional hypopituitarism. This assumption is only correct in very special conditions. Hunger usually only causes a failure of the gonadotropins, which is a sensible economical move. On the other hand, true hypofunction of the thyroid gland cannot be demonstrated in starvation. Although the basal metabolic rate is usually reduced, there is only hypometabolism without hypothyroidism. The capacity to take up iodine, the serum thyroxine, and the histological findings in the thyroid glands of starved subjects are not indicative of hypofunction. The adrenals in man are found to be normal or even hypertrophic. Although steroid excretion is reduced, true adrenal insufficiency cannot be proven. Finally, histological findings in the hypophysis show displacement of the different cells types [deviation to the left and eosinophilia (UEHLINGER, 1948)], which suggest overexertion of the organ rather than hypofunction.

Hunger leads to an illness which is different from true hypopituitarism. Only the unfortunate fact that cachexia was formerly equated with hypopituitarism forced comparisons between this condition and starvation. The impressive term, "hunger-induced pseudohypophysectomy" was coined on the basis of too few convincing animal experiments and should be avoided. Trials with hormone substitution are unsuccessful in these cases, as they are in anorexia nervosa.

8. Course and Prognosis

Since hypophyseal failure is compatible with life, patients with hypopituitarism can live for many years without substitution. It is true, however, that their working capacity decreases and they finally become invalids. The life

expectancy is affected primarily by the development of intercurrent diseases. Resistance to all types of stress is reduced, since the hypophysio-adrenal system no longer functions. Febrile illnesses, vomiting with diarrhea, and minor operations may precipitate a crisis at any time, and the patients may die in coma. The patients become sleepy, fall into stupor and later become comatose, with epileptic fits due to hypoglycemia. In pituitary coma, they are stiff and bent or completely flabby. The pulse is hardly perceptible, there is bradycardia and the heart sounds are very weak. The breathing is slow, often only minimal. There is severe hypothermia and the body temperature may fall as low as 32 °C. Then it can be measured only rectally with special thermometers. The nose and extremities are cold, the skin pale and dry with no trace of prespiration. As in myxedema coma, hyperkapnia is the cause of unconsciousness by way of inhibition of the respiratory center and weakness of the respiratory muscles; thickening of the alveolo-capillary membranes by myxedematous infiltration may also be involved. Hypoglycemia due to inadequate gluconeogenesis and absence of lipolysis caused by growth hormone failure also contribute to the coma. Finally, hypotonia and shock play some part on the coma due to lack of cortisone and therefore ineffective noradrenaline. If the patients fall into coma, the prognosis is unfavorable even now. The therapy is the same as in myxedema coma (see p. 162f.). Untreated patients live for an average of 10–15 years. A few isolated cases have survived for over 40 years. The substitution therapy now available can replace the missing hormones almost completely, so that life expectancy approaches that of healthy individuals when this treatment is given.

9. Therapy

a) Substitution

Substitution with the deficient adenotropic hormones, FSH, LH, TSH, and ACTH is unsuitable for long-term treatment. TSH preparation cease to be effective after long-term administration due to the formation of antibodies. The daily injections of depot-ACTH are tiresome, and substitution of the hormones of the secondary glands is preferable. Adrenal failure must be corrected first of all. A daily oral dose of 12.5–25 mg cortisone acetate makes the patients feel better and more enterprising. The apathy disappears, and the patients recover their interest in life and become more active. Cortisone restores their resistance. Intelligent patients should be taught to adjust their cortisone requirements for themselves. The administration must be greatly increased, 4–8 fold, in physical exertion or in illness. If oral administration is no longer possible due to nausea the patient must consult his doctor at once to obtain i.v. or i.m. injections of cortisone hemisuccinate or water-soluble prednisolone. Substitution of the thyroid hormones is next in importance and should always be started only a few days after the introduction of cortisone therapy. A dose of 0.1–0.3 mg of Na-l-thyroxine compensates the myxedematous features, restores the patient's working capacity, and makes the cold feeling disappear. It is advisable to start with a dose of 0.05 mg and to reach the optimal dose over the course of some weeks or months. In contrast to ADDISON'S disease, it is not necessary to supplement the cortisone with DCA or fluorocortisol, since the adrenals are still capable of maintaining a reduced aldosterone secretion. The crisis in hypopituitarism must be treated basically in the same way as the addisonian crisis (see p. 330f.). When hypothyroidism is pronounced, i.e. when the temperature is subnormal, the condition must be treated as in myxedema coma (see p. 162f.). The same precautions and substitutions are applicable in minor and major surgery as in the case of primary adrenal insufficiency (see p. 122 for substitution therapy during hypophyseal operations).

It must be determined individually for each patient whether gonadal hormones should be given in addition to the thyroid and adrenal substitution treatment. Spontaneous recurrence of menstruation has been observed during cortisone and thyroxine treatment (ENGSTROM, 1961). In general, one should avoid the cyclic use of ovarian hormones. Restoration of menstruation presents a stress to the patient, and should only by considered for psychological reasons. (E.g. 1 mg stilbestrol may be given for 25 days per month.) Long-acting depot testosterone may be given every 4 weeks to male patients. This improves the well-being of the patient and may restore libido and potency. The administration of testosterone to the woman, which may have the unpleasant side effects of virilization, is only justified in special cases. A trial with 2.5–5 mg fluoxymesterone is indicated in cases with loss of libido. The physiological significance of the adrenal androgens on the psyche shows individual variation. There are experienced authors who advocate testosterone therapy in women with hypopituitarism. Since HMG (see p. 600f.), a human gonadotropin with a FSH-effect, has become available, it has become possible to maintain gonadal

function in the woman as well as in the man with HMG and HCG, despite complete hypophyseal failure. Pregnancy and normospermia are thus possible. This treatment is costly in time and material for both doctor and patient, and is only rarely possible. Whether the therapeutic use of effective growth hormone, once it becomes available, will eventually be justified in adult hypopituitarism is questionable. The patient substituted with cortisone, thyroxine, and sex hormones is physically and psychologically fully efficient.

b) Restitution of the Atrophy

In postpartum necrosis, there is only one way of causing the remaining anterior pituitary lobe of the hypophysis to hypertrophy, and it is rarely possible; this is pregnancy. If the patient succeeds in conceiving again (SCHNEEBERG, 1960; MARTIN, 1970) the remainder of the hypophysis shows signs of a physiological hypertrophy which does not regress after the pregnancy. A new pregnancy does not always have this effect, however, and in isolated cases where the disease has not been of long standing, it is difficult to demonstrate that success is due to the pregnancy.

F. Hyperfunction of the Adenohypophysis

1. Classification

Hyperfunction of the pituitary-adrenocortical system is described on p. 332ff. Only prolactin and STH have been shown to produce an illness due to the overproduction of pituitary hormones. See Chap. XIX for the premature, but not excessive, gonadotropin production in pubertas praecox.

For information on reactive pituitary adenomas in hypothyroidism see p. 154, and for a discussion on pituitary adenomas following adrenalectomy in Cushing's syndrome see p. 356.

The overproduction of growth hormone leads to pituitary gigantism if the epiphyseal cartilages are still open, and to acromegaly if the epiphyseal cartilages have fused.

2. Syndrome of Galactorrhea and Amenorrhea with Low Gonadotropin Excretion (AHUMADA-ARGONZ-DEL CASTILLO; FORBES, HENNEMAN, GRISWOLD and ALBRIGHT)

The syndrome of persistent galactorrhea and amenorrhea, in the absence of pregnancy and acromegaly, is due to overproduction of prolactin. A pituitary tumor is detected in about 50% of cases. Prolactin overproduction has been shown to occur in an extirpated tumor (FRIESEN, 1972), and recently, greatly elevated serum prolactin levels have been shown in nearly all patients with this syndrome and other types of galactorrhea. The adenoma, which was classified primarily as chromophobe, contains cells which can possibly be stained specifically. Either it produces too much prolactin, or regulation by the prolactin-inhibiting hypothalamic factor (PIF) is impaired. See p. 30 for persistent lactation with amenorrhea and decreased gonadotropin excretion after pregnancy (Chiari-Frommel syndrome). See p. 592 for glactorrhea induced by neural or pharmacological factors. Of FORBES' 15 patients, 9 had never been pregnant, 8 showed symptoms of a pituitary tumor, and the biopsies revealed a chromophobe adenoma in 3 cases. The symptoms arose between the 20th and 30th years in all the patients. Obesity, hirsutism and seborrhea are almost always present. The levels of 17-ketosteroids are usually slightly elevated; the gonadotropin excretion is reduced or absent, whereas it is normal during amenorrhea caused by lactation. There are signs of estrogen deficiency. In contrast to acromegaly, the serum phosphorus is always normal. No elevation of prolactin serum levels results from arginin infusion or hypoglycemia. A few cases of galactorrhea have been found associated with a craniopharyngioma. In others there have been tumors interrupting the continuity to the hypothalamus (GUINET, 1961). See Chap. XIX for galactorrhea and pubertas praecox in hypothyroidism in childhood.

Galactorrhea can occur during treatment with chlorpromazine, imipramine, reserpine, and ovulatory inhibitors or after pituitary stalk section which is explained by the lack of PIF.

L-Dopa stimulates growth hormone secretion but inhibits that of prolactin. Patients with the Forbes-Albright syndrome and other conditions causing galactorrhea have been treated with L-Dopa which has normalized prolactin and gonadotropin levels (TURKINGTON, 1972; BESSER, 1972) but had inconsistent effects on galactorrhea.

Galactorrhea and hyperprolactinemia are usually accompanied by low or absent gonadotropins, amenorrhea in women and impotence in men. There seems to be a reciprocal mechanism controlling the secretion of prolactin and gonadotropins.

The ergot alkaloid 2-Br-α-ergocryptine is a potent inhibitor of prolactin (FLÜCKIGER, 1968) in normal women as well as in patients with the Forbes-Albright syndrome (DEL POZO, 1973; BESSER, 1972). It acts directly on the

pituitary and not via the hypothalamus (FLÜCKIGER, 1972).

Patients with galactorrhea, high prolactin and low gonadotropin levels, and amenorrhea or impotency were normalized by treatment with 3 to 10 mg brom-ergocryptine p.o. daily. The prolactin and gonadotropin levels normalized after one month, and galactorrhea disappeared and menstruation was restored in the second month. After six months of treatment one patient with the syndrome became pregnant (BESSER, 1972; DEL POZZO, 1973).

Potency was reestablished in one of two men with chromophobe adenoma and hyperprolactinemia (BESSER, 1972).

3. Pituitary Gigantism

A. PRADER and M. ZACHMANN

During growth, an acidophil pituitary adenoma does not give rise to acromegaly but rather to gigantism (Fig. 10). In addition to the gigantism, acromegalic features may arise during the course of puberty, and combinations of gigantism with the complete picture of acromegaly in adulthood are possible even in young patients.

This is an extremely rare disease and possibly occurs more frequently in the male. A pathological acceleration of growth usually arises shortly before or around the onset of puberty. Nevertheless, patients have been described whose symptoms started long before puberty. The youngest patient in whom accelerated growth has been reported was five months old (the Alton giant described by BEHRENS and BARR in 1932).

If left untreated the illness results in symptoms of a deficiency of all adenotropic hormones in addition to the excessive growth hormone production, and usually leads to death in the second or third decade or even earlier. The initial isolated growth acceleration is characteristic. Bone development is not accelerated or only slightly, so that growth may not only be accelerated but also continue for an abnormally long time. Puberty usually follows a normal course at first but may be subject to disorders during the later course due to displacement of the gonadotropin-producing cells. Hypogonadotropic hypogonadism (see p. 469 f.) and eunochoid proportions may result from this. Symptoms of over-production of growth hormone can be replaced by those of progressive insufficiency of all adenotropic hormones over the course of years.

The laboratory findings correspond in general to those in acromegaly in adulthood: serum inorganic phosphorus may be elevated to values over 5–6 mg%. Growth hormone concentration in the plasma has only been investigated by radioimmunological methods, in a few cases, and fasting levels of 100–300 ng/ml were found, about one hundred times higher than normal. It was not possible to produce further stimulation of growth hormone secretion by intravenous injection of insulin. Nor is the plasma concentration reduced by a glucose tolerance test (oral or intravenous). Furthermore, both oral or intravenous glucose tolerance tests and administration of tolbutamide lead to a marked rise in plasma insulin concentration, as in the healthy subject. The blood sugar curve is often pathologic following the glucose tolerance test, being similar to that obtained in frank or chemical diabetes. In contrast to acromegaly in the adult, however, a frank diabetes mellitus is rarely present.

Primordial or constitutional gigantism and cerebral gigantism (SOTOS) are the most important conditions for the differential diagnosis (see Chap. XIX). General signs of elevated intracranial pressure, direct pressure effects of the tumor.

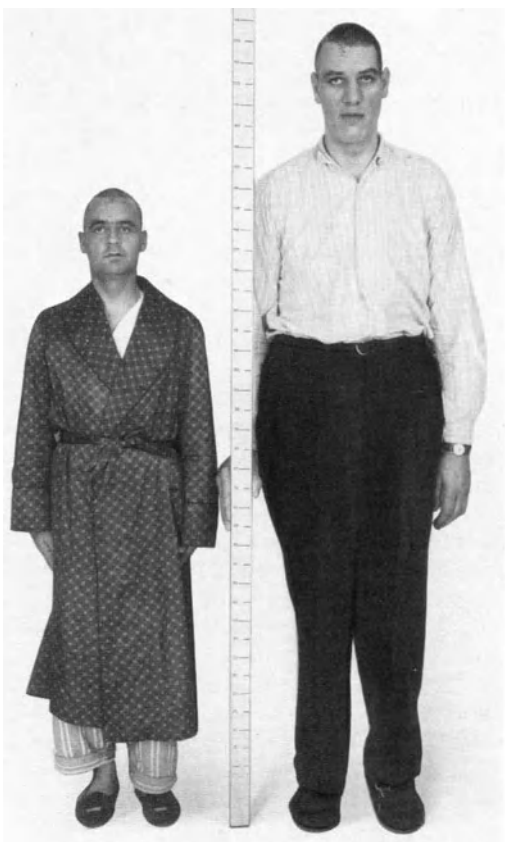


Fig. 10. 24-year-old giant with acromegaly, 210 cm in height and weighing 145 kg, with eosinophilic pituitary adenoma, and increased growth since the 16th year, next to a man 170 cm tall

such as enlargement and extension of the sella (which often arise late), impaired vision, and bitemporal hemianopia are important for the diagnosis. These valuable diagnostic indications may, however, be absent since the tumors are often very small. Diagnosis is then only possible on the basis of the greatly raised growth hormone concentration in the plasma and the blood sugar and insulin estimated during a glucose tolerance test. All these tests yield normal results in other forms of gigantism.

Surgical removal of the tumor or radiation of the hypophysis (externally or by implantation of e.g. yttrium) are possible methods of causal therapy. The therapeutic result, however, is generally disappointing. It is known that in acromegaly in adulthood, high concentrations of growth hormone in the plasma may persist even after surgical removal of the hypophysis. This has also been observed in a patient with pituitary gigantism. The prognosis must therefore be subject to caution even if the tumor appears to have been completely removed. Substitution therapy for the resulting secondary adrenocortical insufficiency and hypothyroidism is often necessary after radical surgery.

4. Acromegaly

a) Occurrence and Incidence

Acromegaly, like all other endocrine diseases except diabetes mellitus and thyroid dysfunction, is a relatively uncommon disease. Statistics from large hospitals show one case of acromegaly in 2000 to 15000 admissions. A total of 36193 patients seen at the Medical Clinic in Zurich in 10 years included 45 cases of acromegaly. About 20% of patients with pituitary tumors develop acromegaly. The disease affects all races and is most common in the 3rd and 4th decades of life, though it may occur at any age.

The disease usually begins soon after puberty and the sexes are equally affected. It has been claimed that 25 cases of this disease have been observed in children under 15 years, but the condition may be confused with constitutional abnormalities. The few reports of familial occurrence may be due to confusion with the constitutional acromegaloid (see p. 114). Repeated reports of the occurrence of acromegaly in only one of homozygous twins make a hereditary factor appear unlikely.

b) Etiology and Pathogenesis

Acromegaly is due to overproduction of growth hormone which can be measured radioimmunologically. The reason for it is almost always a tumor-like growth of the eosinophil cells which produce STH. It is not known for certain whether certain cases of acromegaly can arise from a disorder of the hypothalamic regulation by way of the adenohypophysis, possibly due to tumor formation after acidophilic hypertrophy and hyperplasia caused by chronic overstimulation.

Two types of acromegaly can be differentiated according to the plasma growth hormone. In one type, the overproduction is uninfluenced by metabolic stimuli (see p. 89f.). In the other type, which is much more frequent, the growth hormone level can be made to fall with glucose and be elevated by hypoglycemia, or there may even be a paradoxical rise in growth hormone after a glucose load (LAURENCE, 1970). It is not known whether a different pathogenesis is involved in the latter condition of whether the autonomy is due to lesions of the hypophyseal stalk (GLICK, 1965). These considerations may have some therapeutic implications. There is no clinical differentiation. As a rule, there is some correlation between the size of the sella and the overproduction of growth hormone, but large sellas may be found in the presence of low growth hormone concentrations, and normal-sized sellas may be present in active acromegaly. In the female the disease appears to be most common in the 2nd and 5th decades (SOENKSEN, 1967). The overproduction of growth hormone leads to an increased growth of the skeleton and connective tissues, the musculature, the internal organs, the blood vessels, and the skin. As long as the epiphyseal cartilages are still open the accelerated growth results in gigantism. Acromegaly results after fusion of the epiphyses. Destruction of the remaining cells of the adenohypophysis may result in failure of individual or of all adenotropic hormones, giving rise to the combined picture of acromegaly and hypopituitarism. In a few cases, increased prolactin has also been demonstrated as well as increased growth hormone. The symptom of galactorrhea is probably attributable to the former.

Among the numerous attempts to explain the pathogenesis of acromegaly, KEETH's atavistic hypothesis (ATKINSON), is of speculative interest. It states that acromegaly is a regression into the phylogenetic stages of Neanderthal man or the anthropoids. The development of the extremities, the prognathia and the excessive pneumatization of the sinus are in fact characteristic of these men and animals. LOUIS BOLK's hypothesis of fetalisation considers acromegaly as a regression into a phylogenetically older phase, due to the absence of inhibitory effects which increase with advancing civilization.

c) Pathologic Anatomy

α) Pituitary

Most frequently, acromegaly or gigantism is due to an acidophil tumor of the pituitary. It may include other cells with adenotropic activities. Not infrequently, there may also be a chromophobe adenoma. In the adenomas, however, growth hormone granulas may still be revealed by electron microscopy. The tumor is not necessarily in the anterior hypophysis, but may be in the region of the pharyngeal roof or the sinus sphenoidalis. Sometimes, there is only hyperplasia of the acidophils with no proper tumor formation but with clear-cut enlargement of the hypophysis. Occasionally, the disorder responsible for the uncontrolled STH production has to be sought in an extracranial region (bronchial carcinoma with ectopic hormone production, see Chap. XVI). Acidophil adenomas may reach a considerable size and can destroy the adjacent bony structures, such as the sphenoid and base of the skull. Genuine carcinoma with metastases has only exceptionally been observed. The remaining hypophyseal tissue is compressed and destroyed; the tumor tissue itself is also often affected by necrosis and bleeding. The tumor cells can be very irregular and their nuclei are often of different size. The differentiations and generative polymorphia may thus be pronounced, so that even "hypophyseal sarcomas" are mentioned in earlier literature.

β) Other Endocrine Glands

Hyperplastic processes often occur if the pituitary tissue is not destroyed by the tumor. In about 50% of cases the thyroid gland is enlarged and a diffuse or nodular goiter may develop. The cause may be the growth hormone effect, the increase in TBPA (see Chap. VI, p. 143) or a low inorganic iodine level due to an increase in iodine clearance (MUKHTAR, 1971). The parathyroids may also be enlarged and demonstrate true adenomas. The hyperplasia is possibly due to the elevated serum phosphate level. The adrenal cortex is usually hyperplastic and sometimes contains cortical nodules. The medulla, on the other hand, is not thought to be involved in this enlarging process. Exocrine tissue and pancreatic islands, generally the B-cells, are very active and degranulated. Infiltration by glycogen can also be observed in untreated cases. In the gonads, regressive changes usually predominate, Nevertheless, a few tumors have also been found. Acidophil pituitary adenomas may be combined with other different endocrine tumors; they are often found in the true, hereditary endocrine adenomatosis of the WERMER type (see Chap. XVIII).

γ) Other Organs

The other internal organs also increase in size. Splanchnomegaly results. Involvement of the heart may be excessive, giving rise to myocardial fibrosis. The skeleton is particularly affected. After skeletal maturation is complete, the growth impulse affects mainly the remaining cartilagenous areas and the extremities. The cartilage of the ribs, the marginal border of the vertebral and invertebral discs, and of the joints proliferate. In addition, they show a pronounced tendency to ossify, and even the auricular cartilage shows this feature. The damage to the articular cartilage leads to deforming arthrosis. Hyperostosis and increased bone formation, and to a lesser extent increased bone resorption with osteosclerosis, develop in the skeleton.

These lead to an increased bone width, normal or increased cortical bone but decreased trabecular bone (RIGGS, 1972).

Tendon and muscle insertions are frequently thickened.

d) Clinical Picture and Symptoms

α) History and Psychic Changes

The patient consults his doctor only rarely because of the change in appearance. The acromegalic changes develop so slowly over the course of years that the patient himself hardly notices them, whereas his acquaintances sometimes do. The statement from an adult patient that he suddenly has to buy larger shoes (when flat feet can be excluded) and larger gloves is of great diagnostic value. Persistent headaches are a common symptom and are seldom absent. It is independent of cerebral compression, and the genesis is unknown. Visual disturbances often cause the acromegalic patients to consult the doctor (see p. 112).

Tiredness and muscular weakness only develop after long duration of the illness. They can be the most prevalent complaints. Amenorrhea in the female and loss of libido and potency in the male are almost always present. Pregnancy is, however, possible. Onset of the disease in connection with a pregnancy is not uncommon. Diabetes mellitus (see p. 113f.) occurs in one fifth of acromegalic cases.

The skeletal changes may cause backache. Paresthesia in the form of formication or numbness in the upper extremities are often

present. Their pathogenesis is unexplained. They are not due to hyperostoses in the region of intervertebral foramina. Vasomotor rhinitis is common, often with a secretion tinged with blood. Bulimia, polydipsia, and polyuria are not sufficiently severe to cause the patient to consult the physician.

Galactorrhea due to overproduction of prolactin and STH is uncommon in female patients and extremely rare in male acromegalic patients.

Acromegaly usually leads to profound psychological changes which make up a form of the endocrine psychosyndrome but with a special variation. Contrary to widespread opinion, these changes are not psychoses. There is no relation to schizophrenia, manic depressive psychosis, or epilepsy. The incidence of these psychoses is not higher in the relatives of acromegalic patients than in the normal population (BLICKENSTORFER, 1951). Nor is there any genetic connection between acromegaly and the constitutional anomaly of acromegaloidism (p. 114), in which psychopathy often occurs. The acromegalic character changes are not due to the physical disfiguration alone, and cannot be fully explained by the cerebral compression either. Character changes typical to acromegaly can occur in patients who pay little attention to their disfiguration and do not suffer from it. On the other hand, these changes have been noticed in patients in whom there is no cerebral compression. The psychopathy in acromegaly consists in character changes and episodic changes in mood in association with changes in the sphere of drive. The character changes present as apathy, loss of initiative, lack of motivation, and egocentricity. The patient becomes withdrawn, secluded and odd. Passive cheerfulness can be present one moment, and melancholic resignation and animosity the next. Their affectivity towards the outer world fades, and using the simile of the BLEULER school (KELLER, 1949), "they sound like an orchestra behind a thick curtain". The patients find their inability to communicate subjectively painful and are often depressed at the beginning of the illness. During the course of the illness, they become resigned to their destiny, and ultimately euphoria may often develop. Indifference, fear, and distrust are expressions of long episodes of depression. They are interrupted by bursts of mood changes such as irritability, anger, and restlessness. Sexuality and libido are usually reduced in both sexes, and potency is reduced in the man. The initial increase which is often mentioned is not quite certain. The disappearance of the sexuality is explained by the gonadal failure.

Increases in hunger and thirst can be explained by the metabolic disorders (see p. 113). Contrary to widespread opinion, the intellect is hardly impaired in acromegaly. The bradyphrenia may simulate oligophrenia. These psychological changes are due to growth hormone itself or to metabolic disturbances induced by it. The character changes, once developed, usually remain uninfluenced or admit of only slight improvement. Acromegalic patients often need psychotherapy. The rare patients in whom acromegaly is accompanied by persistent lactation may develop a pronounced maternal instinct in addition to the psychological changes described above (BLEULER, 1964).

β) General Examination

Severe acromegalic disfiguration of a face is so obvious that the diagnosis can be made at first glance (Figs. 11–12). The coarse features give a threatening and sinister appearance, even, when some apathetic placidity is present (Fig. 12). The figures of Punch and Polichinelle-Pulcinella show acromegalic features. The lower jaw is greatly enlarged and the chin protrudes. Prognathism with widely separated teeth develops. The lower incisors come to lie in front of the upper (supraclusion). The large nose is thickened and puffy, and the supra-orbital projections and the zygomatic arches protrude a long way forward. The ears are fleshy and large, and excess skin lies in broad folds on the forehead. The tongue is also involved in the general tissue growth and is deformed and large. It looks like a piece of raw meat and leads to thick speech. The lips, particularly the lower, are large and pendulous.

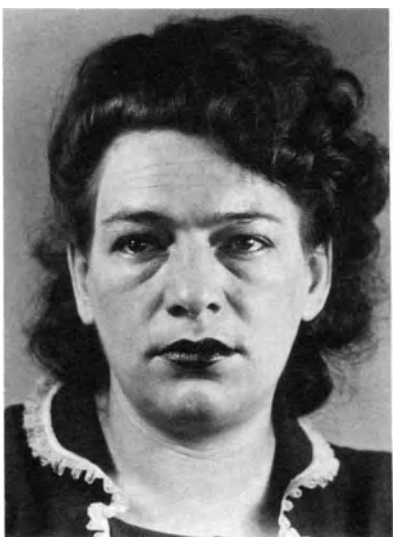


Fig. 11. 38-year-old female patient with acromegaly. Incipient facial changes. Eosinophil pituitary adenoma verified at operation (Prof. KRAYENBÜHL, Neurosurgical Clinic, Zurich)

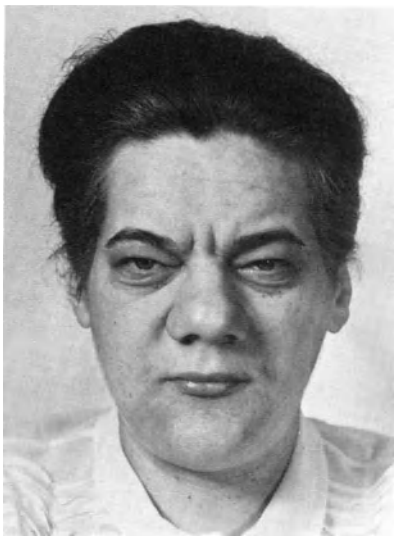


Fig. 12. 35-year-old female patient with acromegaly. Advanced facial changes (Prof. KRAYENBÜHL, Neurosurgical Clinic, Zurich)

A soft tissue type in which the facial changes are predominantly due to growth of the cutis and subcutis must be differentiated from a bony type. Usually both elements contribute to the acromegalic changes. PIERRE MARIE spoke of the type "en carré" or the type "en long"

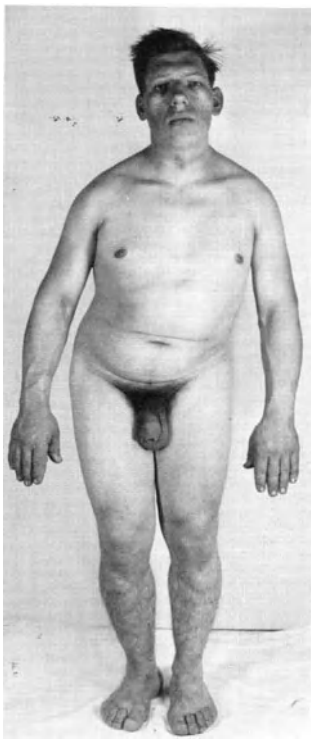


Fig. 13. 29-year-old male patient with acromegaly (Prof. KRAYENBÜHL, Neurosurgical Clinic, Zurich)

depending on the transverse or longitudinal expansion of the face.

After the changes in the face, those in the hands and feet are most striking. The soft tissues are more involved than the skeletal structures. The fingers are sausage-shaped and the hand looks like an animal's paw (Fig. 13).

The kyphotic thoracic vertebral column causes the acromegalic patient to hold his head bent forwards. The barrel-shaped, deep thorax may protrude towards the sternum forming a pigeon chest.

The average height of acromegalic patients exceeds that of the comparable population, probably because the disease usually begins before epiphyseal fusion occurs.

Hyperfunction of the cutaneous appendage organs leads to hyperhidrosis and to seborrhea. Increased pigmentation is found in about half the cases. Sometimes pronounced hirsutism is seen in women due to direct stimulation of the hair follicles by growth hormone. See p. 113 for urinary steroids.

γ) Changes in the Organs

Skeleton: The acromegalic changes in the skeleton are most striking.

The cranial deformity can be recognized externally. All the cranial measurements are increased, including the intracranial capacity. The patients often notice that their hats become too small. The external occipital protuberance and the supraorbital pads are protruding. The cranial sutures are closed. There is almost always diffuse hyperostosis. Localized hyperostosis is less common and is associated with a thickening of the os parietale (LANG, 1961). The frontal sinuses are strikingly large and often extend sidewards. The sella is usually widened with a surface area of 120–230 mm^2 on the X-ray plate. The normal sella, in contrast, is 9–12 mm deep and 12–15 mm long, and has a surface area of 50–120 mm^2. A normal-sized sella can, however, be found in acromegaly. In 6% of cases, it is absolutely intact. It is distended at first, and then if the pituitary grows further suprasellarly, the dorsum of the sella is raised. The anterior and posterior clinoid processes atrophy. There is no connection between the size of the sella and the growth hormone production. See MUNDINGER (1967) for the methods of estimating the size of the sella. The tumors mainly extend towards the sphenoidal sinus and erode the clivus. The dorsum of the sella becomes elongated and also atrophic. The entrance to the sella is often only insignificantly widened, in contrast to the X-ray findings in chromophobe pituitary adenomas.

A distinction between the so-called *Troell-Junet* syndrome and the more usual form of acromegaly is only justified when osteoclasia of the compact bone and osteolysis of the spongiosa are histologically demonstrable as well as the diffuse hyperostosis of the roof of the cranium. Clinical differentiation between this syndrome and acromegaly is not easy. In addition to the acromegalic changes, toxic goiter, diffuse hyperostosis of the roof of the cranium, and diabetes mellitus have also been mentioned.

Growth hormone promotes endochondral and periostal bone growth. The endochondral growth, where still present, is elevated. Inactive foci are reactivated. Therefore, the cartilages of the ribs grow most, causing the ventral expansion of the thorax. The activity of the acromegaly can be assessed from biopsies of the bony-cartilaginous junctions of the ribs. An acromegalic rosary may be formed. The periosteal bone growth produces exostoses, which are most pronounced at the insertions of the ligaments and tendons. In general, the osseous transformation is increased. This can also be shown by means of ^{47}Ca (BELL, 1967). Anabolism usually exceeds the catabolic process. Pneumatization of the frontal and maxillary sinuses and of the mastoid processes increases. There is marked internal bony resorption, whereas externally, appositional bone growth is in progress. This increased transformation produces changes in the bone structure so that osteosclerosis as well as osteoporosis can occur. The osteolytic process can be so pronounced at individual points that cysts are formed, e.g. in the epicondyls. Most of the hyperostoses, those on the lower jaw, at the end phalanges, at the epiphyses, and on the metatarsals, are periostal in origin. However, hypostosis may also occur, so that the metacarpals may either be thickened or become thinner in the cortex. Growth of the cartilage produces irregular protuberances in the region of the joints, which may give rise to a picture similar to that in arthrosis deformans. The changes in the vertrebral bodies are of a different type and are most pronounced in the lower thoracic vertrebrae. New osseous tissue is deposited around the vertebral body like a collar, predominantly ventrally, due partly to reactivation of the cartilaginous border ridges, and partly to periostal growth. The intervertebral discs also grow from the perichondrium. Primitive cartilage is thereby converted into hyaline cartilage. At the same time changes occur in the intervertebral discs, calcification and ossification may arise. Kyphosis of the thoracic vertebral column with compensatory lordosis of the lumbar vertebrae develops. The cartilage tends to become ossified. The rib cartilages can be completely ossified as well as the auricles. The joints also become involved in this cartilaginous change and arthrosis deformans often occurs. Arthrosis in the knee joints may be the patient's main symptom.

The patho-anatomical skeletal changes can be summarized as follows:

1. Increased cartilaginous proliferation. Revival of cartilaginous growth when body growth is complete. This growth impulse is most noticeable in the ribs and articular cartilages. Carpals and tarsals can merge together.
2. Periostosis with thickening of the ligamentous and tendinous insertions.
3. Osteoporosis and osteosclerosis, occasionally in association with true osteolytic processes and cystic formations.
4. Hyperostoses with expansion of individual skeletal areas, especially of the extremities.
5. Hypostosis with rarefaction of individual bones.
6. Articular changes in the form of cartilaginous defects in the region of hypertrophic cartilage, resulting in arthrosis deformans.

Splanchnomegaly. The internal organs are also involved in the generalized growth. The size of the heart and liver are particularly striking. The gastrointestinal tract is longer and its volume is increased. Megacolon may arise. The larynx also grows. The voice is therefore deep and rough. In addition, it has a nasal tone due to the proliferation of the soft tissues in the nose. The vessels are wide and their walls thickened; varicosis is frequent. The lymphatic tissue and thymus are hyperplastic in over 50% of cases. Nodular or diffuse euthyroid goiters of medium size are found in one third of cases. The incidence of larger goiters is no higher than in the normal population.

Circulatory system. In one third of cases, there is hypertension of uncertain pathogenesis with diastolic values of over 100 mm Hg. Cardiac enlargement is not always a feature of the splanchnomegaly, since both degenerative cardiac diseases and infarcts appear to occur more frequently, and the enlargement only indicates dilatation or hypertrophy (HAMWI, 1960).

Nervous system. Impaired vision and loss of visual fields have been observed in 40% of acromegalic cases. Bitemporal hemianopsia is the typical loss of visual fields. Homonymous hemianopsia or concentric narrowing of the visual field and scotomas also occur occasionally. Papilledema is extremely rare. Paralysis of the ocular muscles and other neurological disorders (see p. 109 f.) can occur with the suprasellar expansion and hemorrhages of the tumor

(HOWARD, 1965). Vision and visual fields can, however, also be intact in fully-developed acromegaly. The pathogenesis of the paresthesia has not been explained (see p. 109). Paresis with hyporeflexia due to hypertrophic neuropathy has been described (STEWART, 1966).

e) Metabolic Changes

See p. 123 f. for the inorganic serum phosphorus. Calcium turnover in the skeleton is increased (BELL, 1967).

The positive nitrogen balance during the active phase is of little clinical diagnostic value. Excretion of creatine and creatinine is increased, but this is not very specific. Gonadotropin excretion is usually found to be normal. This does not explain amenorrhea and loss of libido and potency, wich are frequently found. Slight albuminuria is found in 25% of cases, even in the absence of demonstrable renal failure. Glomerular filtration and plasma flow are increased in keeping with the enlarged extracellular space (IKKOS, 1956).

Basal metabolic rate: It is often stated that the BMR is increased in two-thirds of cases. There are usually only slight deviations of +14 to +18% and these do not indicate a diagnosis of hyperthyroidism. Estimation of the BMR in altered stature is problematic, but elevated values are found in such cases whether standards of weight, height, or cell mass are used (IKKOS, 1956) (see p. 240 f.). Occasionally, increases of over 60% have also been found. This is due to a hypermetabolic state in the absence of hyperthyroidism. This has been demonstrated by radioiodine tests, by the failure to respond to antithyroid drugs, and finally, by the histological examination of goiters removed surgically. There are no clinical signs of overactivity. The radioiodine uptake is found to be either normal or slightly elevated (HAMWI, 1960). On the other hand, the ratio 2/48 h uptake is usually raised. The level of thyroxine-binding protein is usually reduced, that of thyroxine-binding albumin raised, and PBI and thyroxine levels may be low. The T3-resin uptake is usually normal (ROTH, 1967). The increased metabolism must therefore be due solely to the increased quantity of growth hormone.

The adrenocortical steroids and their derivatives in the urine are usually normal. Slight elevations are due less to adrenal hyperplasia than to an increase proportional to the total body weight. The increase in response to ACTH and methyrapone is correspondingly normal as a rule (LIM, 1964), as is the inhibition produced with dexamethasone (HAMWI, 1960), unless the illness is combined with CUSHING'S syndrome or an adrenogenital syndrome (MAUTALEN, 1965).

Diabetes mellitus. Frank diabetes is found in 20% of acromegalic cases, and in 10% the glucose tolerance is reduced. The diabetes may be mild or severe. It is usually not very responsive to insulin, and there is no tendency to acidosis.

Table 4. Frequency of the acromegalic symptoms. (After DAVIDOFF)

Acral enlargement	100%
Sella enlargement	93%
Headaches	87%
Menstrual anomalies	87%
Amenorrhea	73%
Elevated BMR	70%
Visual disturbances	62%
Hyperhidrosis	60%
Hypertrichosis	53%
Skin pigmentation	46%
Increase in weight	39%
Loss of libido	38%
Asthenia	33%
Paresthesia	30%
Hypotension (< 120 mm Hg systolic)	30%
Polyphagia	28%
Cutaneous fibromas	27%
Glycosuria	25%
Polydipsia	25%
Goiters	25%
Diabetes	12%
Loss of body hair	7%
Galactorrhea	4%
Breasts underdeveloped	4%

The diabetes arises on average 10 years after the onset of the first acromegalic symptoms. A certain predisposition seems to be necessary in addition to the acromegaly, for the diabetes to become overt, since the incidence of diabetes in relatives of diabetic acromegalics is considerably higher than in those of nondiabetic acromegalics. Three different stages can be distinguished in the development of the diabetes. At first, glucose tolerance is normal. During the glucose tolerance test, insulin release is prompt but excessive. Later, the insulin output is delayed, and glucose tolerance is normal or reduced. Both stages are reversible with successful treatment. In the third stage, the insulin concentration in the blood is already at a maximum during the fasting state and cannot be increased further (SOENKSEN, 1967). Acromegalic patients not reacting to a glucose infusion with increased insulin release may be latent diabetics or prediabetics (LUFT, 1967). The hyposensitivity to insulin is not explained by the increase of free fatty acids (BECK, 1965).

The renal glucose threshold is usually high. The pathogenesis of acromegalic diabetes has been explained by the metabolic properties of

STH. This inhibits glucose absorption by the musculature. As long as sufficient insulin is present for the glucose to be used for tissue synthesis, carbohydrate metabolism is balanced. Diabetes results when the B-cells of the pancreas fail due to constitutional weakness during persistent over-exertion.

Table 5. Synopsis of examinations

1. History
   Changes in appearance: by examining previous photographs.
   Sexual function: loss of libido and potency.
   Complaints: headaches, tiredness, paresthesia, hyperhidrosis.
2. General examination
   a) Alterations in the shape of the head, trunk and extremities.
   b) Body measurements: height, weight, length of the upper and lower body sections, span, diameter of the head, chest and wrist, size of shoes and gloves, possibly a plaster impression of the teeth.
   c) Ophthalmoscopy, examination of vision and of visual fields.
3. X-ray examinations
   Skull, pneumoencephalography, lateral view of the thoracic vertebral column, possibly hands and feet.
4. Laboratory investigations
   Serum phosphorus, sugar and protein in the urine, fasting blood-sugar and if normal, glucose tolerance test. BMR, if elevated, radio-iodine test, thyroxine, estimation of growth hormone during the insulin or glucose tolerance test.

f) Course and Prognosis, Special Forms

The onset is insidious, although acute forms of the disease with a course of 2–4 years have been described. The symptoms arise in three groups. Excessive growth first becomes apparent and is followed by pressure symptoms of the pituitary tumor, especially those involving the optic nerve and chiasma; symptoms related to the circulatory organs and metabolic disturbances are the last to arise (diabetes, polyphagia, polyuria).

The life expectancy in untreated acromegaly is 13 years on average. The cause of death is most often cerebral compression and hemorrhage in the tumor. Diabetic complications are second in frequency as the cause of death, and the third most frequent cause is cardiac failure in the presence of a hypertrophic but functionally inferior myocardium. The illness can become stationary at any time, and can also have an intermittent course. Finally, hyperpituitarism can be converted into hypopituitarism, where the tumor destroys the remaining pituitary tissue and ultimately degenerates. The signs of hypopituitarism may then be superimposed on the clinical picture of acromegaly (see p. 93). Tiredness, apathy, hypogonadism, hypothyroidism, and finally adrenocortical insufficiency complete the picture. These forms are described as burnt-out acromegaly. Forms of acromegaly combined with thyrotoxicosis, CUSHING's syndrome, neurofibromatosis, or amyotrophic lateral sclerosis have also been described. The acromegalic changes can be limited to one side of the body or to one organ, such as the tongue or the big toe. Such presentations can only be explained by a different response of the endorgans to STH.

BAILY and CUSHING described a case of "fugitive acromegaly", an intermediate form between true acromegaly and the chromophobe adenoma. In the clinical histories of 6 cases there was not a single symptom pointing to hyperfunction of the anterior pituitary lobe. The acromegaloid features appear to have been constitutional anomalies.

g) Differential Diagnosis

The diagnosis presents no difficulties in fully-developed acromegaly when it can be established that the patient's appearance has changed. The condition can, however, be extremely difficult to differentiate from an acromegalic constitution, so-called acromegaloidism. The appearance of the acromegaloid subject can be very similar to that of the acromegalic, although the soft-tissue thickening is never so pronounced. Proof that the patient has changed in appearance is important in the diagnosis of acromegaly. Earlier photographs are especially valuable. The acromegaloid with mild to pronounced characteristic features usually displayed these features even as a child (Fig. 14). The sella is not enlarged, and there are no metabolic deviations. It is not an illness but a constitutional anomaly. Nor is there any genetic connection between acromegaly and acromegaloidism, which does not occur more often in the families of acromegalics than in the normal population.

Acromegalic features occur in cerebral gigantism (SOTOS) and in idiopathic gingival fibromatosis and hypertrichosis (GORLIN, 1964).

The differential diagnosis can also include Paget's disease due to the cranial changes, hypertrophic osteoarthropathy with periostial osseous proliferations, pachydermoperiostosis, and finally, leprosy with leonine facies. Differentiation from myxedema should hardly present any difficulties though it was included in the differential diagnosis by PIERRE MARIE.

Usually difficulty is not encountered in the diagnosis of acromegaly but in deciding whether the disease is still active or has come to a stand-

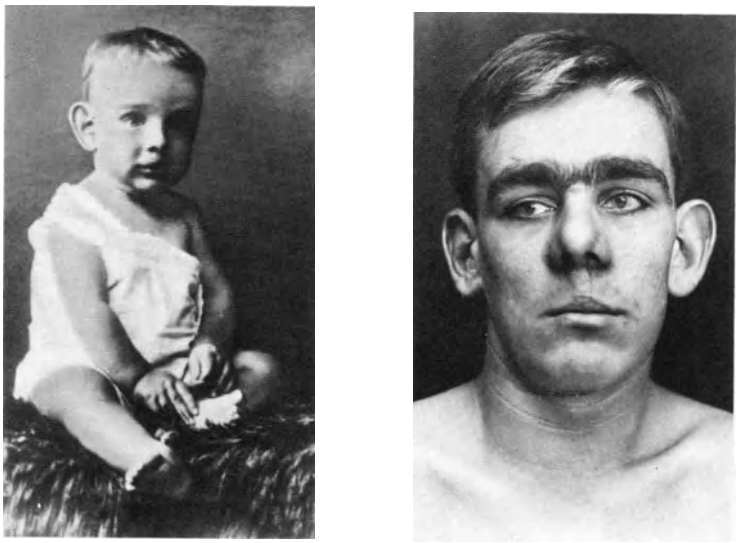


Fig. 14. 23-year-old male patient of acromegaloid constitution. Acromegaloid features already detectable in babyhood (Prof. SCHÜPBACH, Inselspital, Bern)

still. This question is not answered by the appearance, and the history is often no help since growth is slow. The detection of headaches, severe sweating, polydipsia, polyphagia and a raised BMR are of importance. An elevated serum phosphorus is a finding of value since it may indicate an overproduction of growth hormone and becomes normal again with the inactivation of the process. Normal phosphate values do not, however, exclude activity. The activity can best be assessed by measuring the growth hormone during the insulin and glucose tolerance tests. The concentration of HGH and the severity of the illness are not always compatible.

h) Treatment of Acromegaly

Treatment of acromegaly must be directed at the following 3 symptom groups:

1. neurological disorders, especially loss of visual field, and headaches;
2. subjective and objective symptoms of endocrine hyperfunction;
3. concomitant endocrine failure.

The therapeutic problems have been solved only for the 3rd group of symptoms (see p. 105 f.). The degree of success with neurological disorders is very much dependent on the extent of the tumor. The prognosis for neurological disorders is good after surgical removal of a tumor limited chiefly to the sella when it has been diagnosed early. The problem of how to treat the endocrine hyperfunction has not yet been satisfactorily solved (KOZAK, 1966). The symptoms may regress following radiation, and tumor growth may be arrested. Objective remissions can only be demonstrated by the fall or normalization of plasma growth hormone and have not been successfully achieved with conventional or high-voltage irradiation, or with implantation of ^{90}Yt whether or not combined with ^{192}Ir, and not always by operation (GLICK, 1965; LINFOOT, 1965; ROTH, 1968). Even in the so-called burnt-out cases, where there is a clinical remission, growth hormone may still be increased (GREENWOOD, 1965). A final evaluation of the therapy will not be possible until large comparative series of growth hormone measurements are available. In general there is a tendency to radical treatment, even when hypopituitarism must be expected.

There are now six possible methods of treatment.

α) Surgical Removal of the Tumor

This procedure is indicated (1) if neurological disorders are present or (2) if the symptoms do not regress after irradiation or the objective symptoms progress. The neurosurgical transfrontal approach (RAY, 1964) and the rhinological transethmoidal-paranasal approach are both possible (NAGER, 1936; GUIOT, 1958; HAMBERGER, 1960; ESCHER, 1965). More recently, a microsurgical transnasal-transphenoidal approach has been introduced (HARDY, 1969; 1973). The transfrontal approach should be considered when there is suprasellar extension of the tumor and when the neurological failure is extensive as it gives the necessary better vision. When only endocrine hyperfunctional symptoms have to be treated, the transphenoidal operation is

less of a strain to the patient. Meningitis and liquorrhea have been observed occasionally as complications of the rhinological transphenoidal procedure.

If surgery is indicated and the surgeon and patient are agreed, removal of the tumor must be as nearly as possible complete. The tumor is in a capsule and is usually curetted out with a blunt scalpel and sucked off. The capsule in the suprasellar region is resected and that in the sella is left untouched. When the tumor is poorly demarcated total removal of the adenohypophysis must be considered, since it might otherwise infiltrate into the healthy tissue (RAY, 1964). This procedure involves the danger of failure and a higher risk or recurrences if carried out too sparingly. Postoperative irradiation is usually recommended. In 75–80% of the patients endocrine substitution therapy is required postoperatively or after postoperative irradiation (RAY, 1914; ELKINGTON, 1967). Diabetes insipidus is only present when the hypothalamic nuclei are involved and generally does not occur after the removal of the eosinophil adenoma, in contrast to the situation following operations for craniopharyngiomas. Temporary polyuria of different types (see p. 52f.) has been seen when the posterior lobe has been injured (RANDALL, 1960). We recommend prophylactic substitution (see p. 122) for every operation on the diseased pituitary since a latent insufficiency, which can never be excluded, can become manifest under the stress of surgery.

The operative risk is greatly dependent on the extent of the tumor (HEIMBACH, 1964; MARGUTH, 1964). There is practivally no operative mortality today for tumors restricted to the sella. The risk increases with the extent of the tumor, and tumors are inoperable beyond a certain size.

β) Irradiation

X-ray therapy places no great stress on the patient, and there is no mortality. Eosinophil pituitary tumors are relatively radiosensitive, more so than the chromophobe adenoma and the craniopharyngioma. High-voltage therapy (cobalt, betatron) is preferable. The dose must be high, but the surrounding tissues must be protected by applying irradiation from 4–6 fields. Pendulum irradiation can be used or the patient rotated. The X-rays are applied once daily for 2–4 weeks. In order to prevent damage to the brain and optic nerves, the dose to the hypophysis must be kept below 4000 rad.

The patient's symptoms may regress following irradiation. Objective remissions of the endocrine hyperfunction occur. In one large series the elevated plasma growth hormone was unaffected (GLICK, 1965), while in another series a considerable fall was seen in the growth hormone level (ROTH, 1970; LAWRENCE, 1971). Loss of visual fields can regress, but often only after a preliminary temporary deterioration. Impaired vision is an indication for surgery. Nonetheless, when only subjective symptoms are of importance, a trial with irradiation can be tried first. When no definite success is achieved after 2 months, surgery can then be considered. Radiation arachnoiditis hardly ever presents a problem.

γ) Proton-Beams and α-Particle Irradiation

The measurable limits of accelerated atomic nuclei with their slight diffusion, the narrowly restricted Bragg-effect, can produce sharply localized necroses without endangering the surrounding tissues. Complete pituitary elimination can be achieved by this method. Experience will show to what extent it is applicable in the treatment of pituitary tumors. The method is dependent on large atomic acceleration plants which are available for medical purposes in only a few centers, so that only carefully selected patients can be considered (LAWRENCE, 1963, 1965, 1970).

δ) Isotope Implantations

Stereotactic methods (MUNDINGER, 1967) are used to implant several rods or pellets of beta-radiating yttrium^{90} into the hypophysis via the transethmoidal, the transnasal, or the transfrontal route. The yttrium may possibly be combined with iridium^{192}. A dose of 100000 rads is used for complete pituitary elimination and 20000 rads for treating the tumor. This method with yttrium^{90} involves a relatively slight stress for the patient. This method does not have a great future since success is too uncertain, recurrences occur too often, and the complications are too severe. Meningitis, pituitary abscesses, delayed development of liquor fistulas, and lesions of the optic nerves are the most serious disadvantages of this method (GREENWOOD, 1965).

ε) Stereotactic High Frequency Electro-Coagulation

Bipolar high frequency electro-coagulation involves little stress for the patients and can bring about complete elimination of the intrasellar pituitary. Temporary complications rarely develop. The borehole is closed with muscular grafts (ZERVAS, 1965).

ζ) Cryohypophysectomy

It has been clamed that complete pituitary elimination can be achieved by stereotactic infliction of 2–6 deliberate lesions with a local temperature of −180°. Side effects such as diabetes insipidus and visual disturbances are thought to be only temporary (RAND, 1964). The use of this method for tumors must be further investigated. Until now, it has been possible to normalize growth hormone in acromegaly only in some cases, and complications can occur (DASKE, 1966).

ϑ) Treatment with Drugs

Although success has been reported with estrogens, particularly in extremely high doses, in the treatment of acromegaly (ALMQUIST, 1961; MCCULLAGH, 1955), the results are not convincing. Estrogens do not lower the plasma growth hormone; however, they do have a certain inhibitory effect on the periphery. Medroxyprogesterone-acetate in a dose of 40 mg daily, lowers the growth hormone level and may improve the clinical symptoms (LAWRENCE, 1970). Chlorpromazine has the same effect, which suggests the disease is hypothalamic in origin (KOLODNY, 1971). It remains to be seen how reliable these treatments will be. There was an attractive idea that human growth hormone changed slightly by extraction might used to produce antibodies in acromegalic patients; it was hoped that these antibodies would neutralize the patients' own growth hormone but an attempt at therapy based on this idea proved unsuccessful (PRADER, 1966). "Hypophyseostatics" with tolerable toxicity (KOVACS, 1964) are still not available. Hydroxypropiophenone has proved to be ineffective. GRIH (somatostatin) has acute effects (HALL, 1973).

η) Indications for the Choice of Therapy

Providing there are no vital contraindications, the transfrontal or transethmoidal removal of the tumor is indicated in the presence of neurological disturbances, particularly the loss of visual fields. Pressure on the chiasma can thus be eliminated. Whether the transfrontal or the transphenoidal route or both have to be chosen depends on the suprasellar extent of the tumor seen by pneumoencephalography. Other neurological failures, such as paralysis of the abducent and oculomotor nerves resulting particularly from hemorrhage into the tumor can only be counteracted by immediate surgical intervention. Regression of loss of the visual fields can also be achieved with irradiation and with yttrium implantation (MOLINATTI, 1962). As a rule, an interval of 2 to 3 months is necessary before the success of the treatment can be assessed. In each case, however, the decision will be made individually according to the intra- and especially the suprasellar extent of the tumor, the age and general condition of the patient, the nature of the symptoms, and to whether there is concomitant endocrine failure. Surgery is indicated when irradiation produces no success within 2 months.

The object of treatment is firstly to correct the neurological failure or to at least prevent its progress, and secondly to relieve the subjective symptoms, such as headaches, hyperhidrosis, paresthesia and psychic effects, which can sometimes be very severe. The changes in the soft tissues sometimes regress considerably with successful treatment (ROTH, 1967). Regression of the osseous deformities has also been reported, but in practice, this can hardly be expected. Apparent endocrine failure is irreversible almost without exception and must be substituted. Diabetes mellitus of short duration can regress, whereas diabetes mellitus of longer duration is only alleviated or remains uninfluenced.

G. Hormonal-Inactive Pituitary Tumors

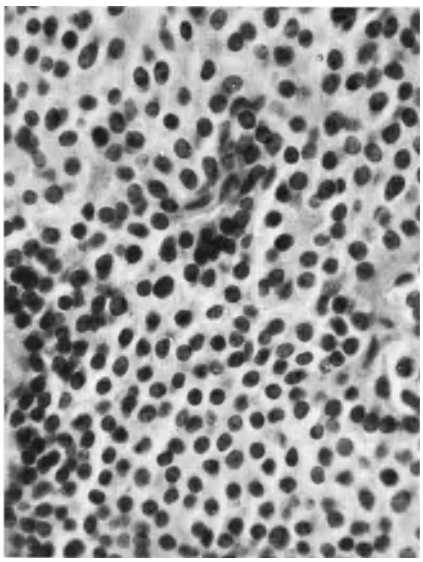
1. Pathological Anatomy

a) Chromophobe Adenomas

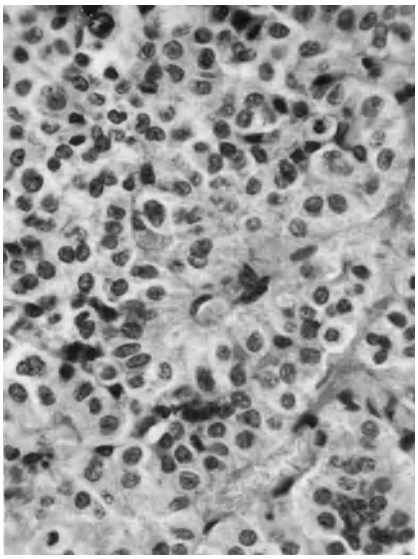
Different statistics show that these tumors account for 6–7% of all brain tumors. They are the most common type of pituitary tumors. Small adenomas, which are only demonstrable with systematic microscopic examination of the pituitary, are much more frequent. The adenomas may remain restricted to the sella, but it is not unusual for them to invade the surrounding structures and they advance relatively often into the base of the brain. True metastases seldom occur. Alveolar, trabecular and papillary adenomas can be differentiated according to the cell arrangements; and the so-called fetal adenomas, the clear-cell, the small-cell, and the cylinder-cell adenomas can be distinguished by the cytology. Regressive changes, particularly cystic formations, are common. On the other hand, calcifications are rare. The endocrine significance of chromophobe adenomas has increased considerably in the last few years. As already mentioned, the chromophobe cell state may reflect a particular endocrine activity, since the cells do not store their secretion, thereby becoming chromophil, but secrete it continually.

b) Cysts

Small cysts containing colloid are particularly common in the marginal area between the anterior and posterior lobes but are of no importance. The large cysts, on the other hand, are of clinical importance. They may be intra- or suprasellar or involve both sections. They may be ciliated epithelium cysts with mucus cells or pavement epithelium cysts. Simple epidermoid cysts are only lined by pavement epithelium, whereas demoid cysts also have cutaneous appendages. Ciliated epithelium cysts contain fluid and occasionally mucus. Pavement epithelium cysts, on the other hand, contain crumbly or mushy substances. Devitalized areas may release a foreign-body reaction. Calcifications and ossifications have been observed, especially in dermoid cysts.



a



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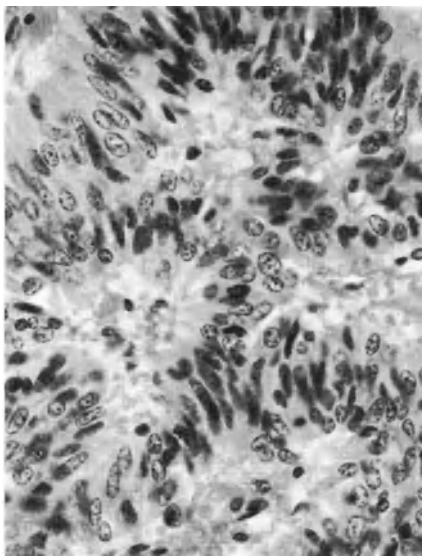

c

Fig. 15 a–c. Chromophobe pituitary adenoma. a) MB 4001/55. 31-year-old woman. Small cell adenoma. b) S 1063/51, 37-year-old man, more like a large-cell adenoma. c) S 932/49, 29-year-old man, the so-called fetal adenoma with high cylinder cells. HE staining, 400 : 1

c) Craniopharyngiomas

These tumors are also occasionally described as adamantinomas or as Erdheim tumors. Like the cysts, they too can be extra- or intrasellar. The tumors are occasionally partly solid but are usually composed of multilocular cysts. They occasionally form an almost unilocular cyst which may attain considerable proportions. The histology shows that the tumors consist of epitheloid cellular rods. In the periphery of these, the cells are palisade-like and are arranged in rows, whereas in the center more network-like structures and cysts are found. Calcification and regressive changes are usually pronounced. The tumors grow slowly, but may attain considerable size, destroying the surrounding tissue and the pituitary in particular.

d) Teratomas

Teratomas may exceptionally occur in the pituitary region. They affect the epiphyseal areas much more frequently, however.

e) Metastases

The posterior lobe is most affected by metastases, which are common in some forms of tumors. Carcinomas of the breast and bronchus tend to metastasize particularly in the hypophysis. Carcinoma of the stomach then follows. Usually the metastasizing tumor dominates the clinical picture to such an extent that signs of pituitary failure hardly become manifest. Diabetes insipidus is, however, not so uncommon in metastasizing carcinomas of the breast.

2. Clinical Features

a) Endocrine-Inactive Chromophobe Adenoma

In contrast to the eosinophil adenoma, the chromophobe adenoma does not often produce headaches. These are caused by pressure on the hypophyseal capsule or by expansion of the tumor into the hypothalamus with compression of the 3rd ventricle, giving rise to occlusive hydrocephalus. Impaired vision is common. At first, the blind spot enlarges, and color vision, especially for red, decreases in the periphery.

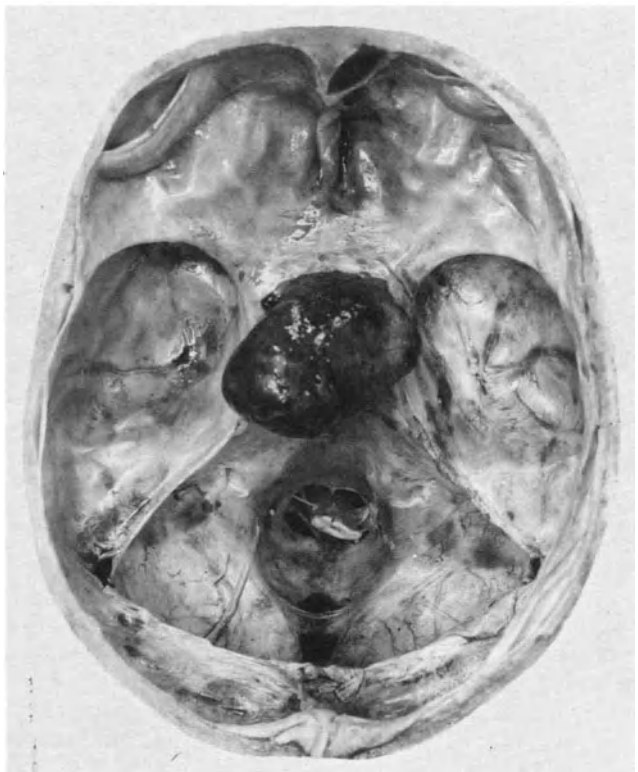

Fig. 16. P. 1024/35, 45-year-old man, chromophobe adenoma of the anterior pituitary lobe protruding from the sella in the shape of a mushroom (view of the base of the skull from above)

The upper temporal quadrant is the first to be lost, followed by the lower temporal. Bitemporal hemianopsia develops, which is often asymmetrical, one eye usually being more affected than the other. Homonymous hemianopsia of the optic tract has also been observed in the adult. Vision decreases and central scotomata develop. Primary optical atrophy develops, leading to complete blindness first in one eye and eventually in both. Paralysis of the ocular muscles can arise, particularly those involving the III and IV cranial nerves. In two-thirds of all cases there is suprasellar expansion whose extent can be certified by a pneumoencephalogram. Pneumoencephalography should be performed in every case of enlarged sella for this reason, and also to exclude the "empty sella syndrome". All possible neurological disorders may then develop, in addition to visual disturbances. These include palsies, hyperreflexia, ataxia, paracusis and deafness. The risk involved in surgery increases with suprasellar expansion, and the operability decreases (HEIMBACH, 1959). Hypothalamic invasion by the adenoma results in diabetes insipidus, somnolence, and disorders in temperature regulation. The protein in the cerebrospinal fluid may be increased. When the suprasellar tumor expands into the 3rd ventricle, occlusive hydrocephalus develops. This may become manifest in papilledema. The X-rays always show a widened sella, ranging in shape between a bowl and a balloon. The entrance of the sella is widened, the dorsum sellae is often raised and usually more or less destroyed. All these symptoms, headache, paralysis of the occular muscles, visual loss, collapse, stupor and coma, can develop in hours. They can be explained by hypophyseal "apoplexy", i.e. spontaneous or traumatic hemorrhage into the tumor. The cerebrospinal fluid may be strongly hemorrhagic or only slightly tinged, and the cell count and protein may be increased. Immediate massive substitution therapy and neurosurgery to relieve pressure can save vision and life (LOCKE, 1961). Localized hemorrhages cause partial endocrine insufficiency and may regress spontaneously (WRIGHT, 1965). Aneurysms, especially of A. carotis interna, may mimic the chromophobe adenoma radiologically and endocrinologically (WHITE, 1961). The slow onset of symptoms often allows the diagnosis only after 3, 5 or 7 years, depending upon whether problems of vision, headaches, or endocrine insufficiency predominate (FUERST, 1966). In an enlarged sella pneumoencephalography may occasionally reveal an empty sella syndrome (KAPLAN, 1969). (See also p. 103f.).

Endocrine symptoms: Endocrine-active tumors are encountered among the chromophobe tumors, and give rise to symptoms of Cushing's syndrome (see p. 344), acromegaly (see p. 109), galactorrhea, amenorrhea and very occasionally to symptons of hyperthyroidsm (GUBLER, 1963; LAMBERG, 1969). LAMBERG has shown that this rare form of hyperthyroidsm (KAPPELER, 1959) is due to TSH overproduction. As a rule, however, chromophobe adenomas are endocrine-inactive. Pressure and compression of the active cells can, but need not, give rise to partial or complete pituitary failure. The chromophobe adenoma leads to overt endocrine failure in one third of cases, and to diminished reserves with preserved basal secretion in one third, while in the remaining third endocrine function is unaffected. Growth hormone and gonadotropin secretion is the most frequently disturbed, followed by ACTH and TSH secretion (NIMAN, 1967). Insufficiency of the posterior lobe with diabetes insipidus has rarely been observed in the chromophobe adenoma.

b) Craniopharyngioma

A craniopharyngioma can occur at any age but shows a predisposition for the young, under 20 years of age, whereas pituitary adenomas occur almost without exception after the 20th year. The craniopharyngioma often contains large cysts. Depending on the direction of growth, visual disturbances or occlusive hydrocephalus due to obstruction of the 3rd ventricle may develop. Symptoms of endocrine failure can also arise due to destruction of the hypothalamic nuclei. The clinical picture is different in the various age groups. In the infant the suprasellar tumor, which usually involves the 3rd ventricle, interrupts the cerebrospinal fluid circulation and blindness results from compression of the chiasma. During puberty, physical development of most patients is retarded. Occlusive hydrocephalus is rare in this age group. Beyond 20 years, the chiasma syndrome dominates the clinical picture, usually combined with sexual dysfunction and disturbances in water equilibrium (diabetes insipidus, adipsia). Supra- and intrasellar calcifications on the X-rays are suggestive of a craniopharyngioma (Fig. 7). They can sometimes appear as mottling and sometimes as bowl-shaped above the sella. Occasionally, a calcified cystic wall can be demonstrated. The sella is often not extended but may be.

Endocrine symptoms: The craniopharyngioma gives rise to destruction of the hypothalamic nuclei with impaired formation and release of the neurohormones, with the result that regulation of the secretion of the anterior pituitary hormones is also impaired. Diabetes

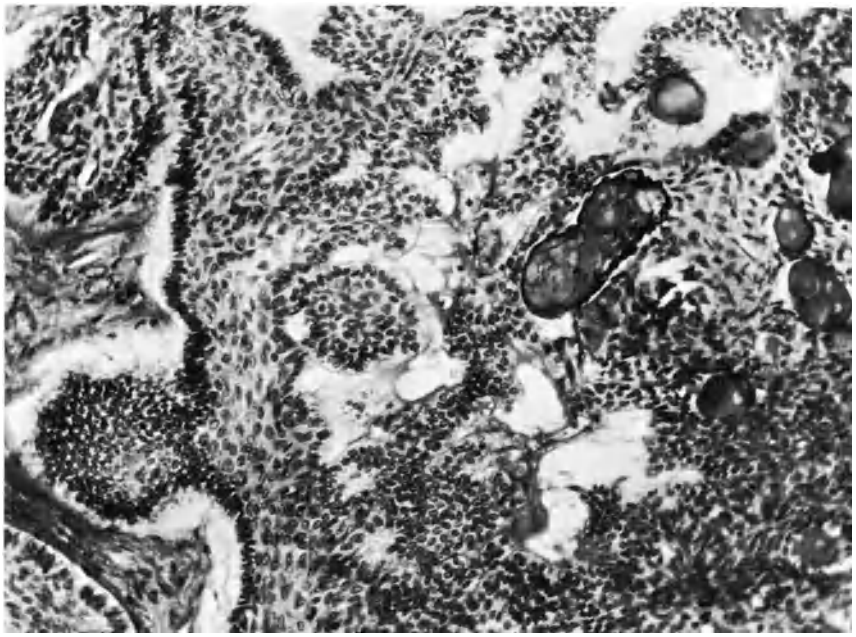

Fig. 17. S 774/52, 8-year-old boy. Craniophryngioma with large calcium inclusions (right of picture). HE staining, 300:1

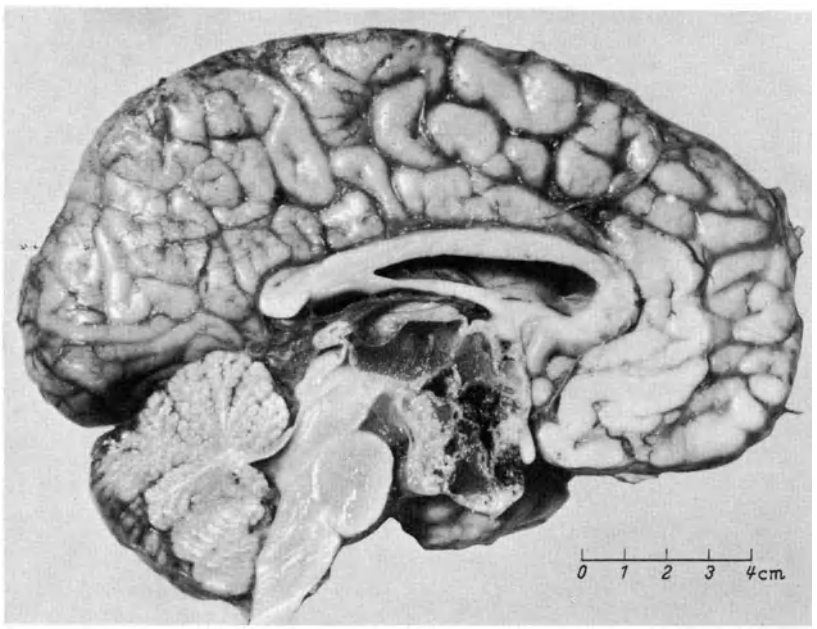

Fig. 18. S 774/52, 8-year-old boy. Craniopharyngioma at the base of the brain with adhesions to the region of the 3rd ventricle

insipidus is common. Growth and maturation are retarded. Hypogonadism occurs frequently, whereas secondary hypothyroidism and secondary adrenal insufficiency have been less frequently observed. Pubertas praecox occurs in a few cases, probably due to the stimulation of hypothalamic centers. See p. 106f. for galactorrhea. Adipsia (see p. 55f.) with chronic hypernatremia has been observed (MATSON, 1964; RICHTER, 1970). The endocrine failure is usually more pronounced after surgerey even if the operations are successful from the neurological aspect (KRAYENBÜHL, 1962).

3. Therapy

Neurosurgical removal of the tumor and X-ray irradiation can be considered in treatment of the chromophobe adenoma. Chromophobe adenomas are not very radiosensitive. 4000–6000 r must be applied to the focus within 30 days. Good results are thought to be obtained with high doses of ultra-hard irradiation (4000 r at 2000 kV) in more than 50% of cases but it is not unusual for the adenomas to be cystic and therefore radiorefractory. They are also usually well-defined and can in the main be removed surgically providing there is no great suprasellar expansion. No time should be lost in trying irradiation in loss of visual fields. The therapy which gives the best results at present is radical surgical removal of the tumor followed by irradiation. As stated on p. 115, the slight endocrine failure, which can be substituted, is more acceptable than the risk of limited success or recurrences.

Table 6. Substitution therapy for operations on the hypophysis

1. At the beginning of anesthesia slow infusion of 1 liter 2 : 1 physiologic glucose/physiologic NaCl solution with 50 mg cortisol hemisuccinate given over 8 hours. A further liter of 2 : 1 physiologic glucose to physiologic NaCl with 50 mg cortisol hemisuccinate given over the next 16 hours.
2. Measure fluid intake and urinary output for 24 hours. Specific gravity and, if possible, body weight to be checked daily. To prevent dehydration due to postoperative diabetes insipidus, the fluid intake should be approximately 0.5 liter more than the urinary output. The body weight should remain reasonably constant. Hematocrit, blood electrolytes and proteins must be estimated in doubtful cases and in unexplained fever (thirst fever). Fluid administration to be adjusted to the findings.
3. On the 1st postoperative day, if the patient can swallow, one 25 mg tablet of cortisone acetate morning and evening. When swallowing is not possible, slow infusion of 1 liter 2 : 1 physiologic glucose/physiologic NaCl solution with 50 mg cortisol hemisuccinate.
   One half 25 mg tablet of cortisone acetate is given morning and afternoon from the 2nd postoperative day.
4. Depending on the course of the illness, all substitution is withdrawn after 1–3 weeks but preferably after 3 months, possibly in hospital. Adrenal, thyroid, and gonadal function is examined. Long-term substitution therapy is decided upon in the presence of complete failure of the anterior lobe of the pituitary. This usually consists of:
   $2 \times {}^1/_2$ tab. cortisone acetate and
   0.2 mg Na-l-thyroxine
   250 mg Depot-Testosterone i.m., monthly in male patients.
   Pediatric doses:
   at 8 years: $^1/_2$ the adult dose
   at 2 years: $^1/_4$ the adult dose

NB: Oral cortisone or intravenous cortisol is preferable to prednisolone or prednisone because of the slight sodium-retaining effect. Prednisolone or prednisone can be used in a $^1/_4$ dose when cortisol and cortisone are unavailable.

Craniopharyngiomas are practically insensitive to radiation. Radical surgical removal of these benign, well-defined tumors involves a high mortality due to the damage which may be expected in the surrounding areas of the brain. Often, only palliative operations achieving removal or partial resection of the very large cysts occasionally present are possible. Palliative treatment of the occlusive hydrocephalus resulting from the craniopharyngioma may become necessary. As a rule, the Torkildsen operation is indicated, in which the cerebrospinal fluid circulation is restored by the insertion of a rubber or polyethylene tube leading to ventriculo-cisternal or ventriculo-atrial shunts. The tube overcomes the obstruction and establishes free communication. Whether hypopituitarism is demonstrable or not preoperatively, extirpation of a pituitary adenoma or of a craniopharyngioma must, as a matter of precaution, be carried out under complete substitution therapy. Table 6 gives the procedure to be adopted.

Almost 50% of patients with pituitary tumors treated surgically or with radiation have clinically apparent mild or pronounced pituitary insufficiency. More thorough investigation reveals failure in four-fifth of cases. See p. 52f. for postoperative diabetes insipidus and polyuria. Nevertheless, only one third of the cases in a large investigation series received substitution therapy. The hormones of the deficient glands should be substituted without hesitation if even the slightest symptoms appear (ELKINGTON, 1967). If no endocrine disorders are demonstrated, a gradual reduction of the substitution therapy should be tried 1–3 weeks postoperatively at the earliest. As a rule, however, a check-up should be undertaken in the hospital 3 months after the operation to decide whether pituitary function has remained intact or recovered after the relieve by the operation.

4. Diagnosis of the Anterior Pituitary Function

The function of the anterior pituitary can be assessed largely by the function of the 3 endocrine glands under its control. Normal function of the gonads, the thyroid gland, and the adrenal cortex indicate normal anterior pituitary function. Thus, pituitary insufficiency is unlikely in a woman with normal menstruation. In the male, the sperm count is a sensitive indication of pituitary function since pituitary insufficiency causes an immediate fall.

Estimation of the adrenotropic hormones and the tests for pituitary reserve have been discussed in the chapters dealing with the individual organs.

Direct and Indirect Estimation of Growth Hormone Activity

a) Biological Determination

Biological methods (GEMZELL, 1959) permitted the detection of only a very high concentration of growth hormone in the plasma, whereas the introduction of the radioimmunological methods for peptide hormones has made it possible to estimate normal basal values and daily variations. Since then estimation of the growth hormone in the plasma has only become significant in the diagnosis of active acromegaly.

b) Radioimmunological Determination

The radioimmunological methods of estimation are described in detail under the method for insulin, whose estimation was first achieved by BERSON and YALLOW (see p. 814). These methods exploit the property of endogenous growth hormone of competitively inhibiting the combination of growth hormone labeled with ^{131}I with specific antibodies. Growth hormone of the added plasma is deduced from the ratio of the free labeled growth hormone to that bound to the antibodies. Separation of the free from the antibody-bound growth hormone is achieved either by electrophoresis (GLICK, 1963; HUNTER, 1964) or by precipitation of the growth hormone bound to the antibodies with antigamma globulin serum (double-antibody precipitation method) and subsequent centrifugation (UTIGER, 1962; BODEN, 1967). These methods can detect concentrations of 0.5 ng/ml, and all give consistent normal values of 0–3 ng/ml after 12 hours' fasting.

With certain reservations, the plasma concentration of growth hormone measured radioimmunologically is the most specific method for evaluation of the growth hormone secretion. This is true providing a flawless radioimmunological method can detect values around 1 ng/ml. Normal adult values after 12–14 hours of fasting and absolute rest lie between 1 and 3 ng/ml. Values of 8–103 ng/ml are found in acromegaly (BODEN, 1967). Elevated values, however, are not definite proof of acromegaly, since any physical task, and probably emotional stress as well, can cause a ten-fold or more increase. In addition, a few values within the normal range have been found in acromegaly. Considerable diurnal variations occur (CRYER, 1969). Also, the low fasting level permits no differentiation between the normal state and pituitary insufficiency. Therefore, only the stimulation or inhibition tests permit definite differentiation.

c) Stimulation Tests

Stimulus for the release of growth hormone can be provoked by 0.1 U/kg body weight (4 U/m^2 body surface) insulin or in insulin-insensitive acromegalics, 0.3 U/kg body weight i.v. Hypoglycemia is still the most reliable, but must consist in a fall of about 50%. Blood sugar and growth hormone must be determined at 0, 15, 30 to 90 and 120 minutes (FRANTZ, 1964; CAPLAN, 1968). Alternatively, 1 g of tolbutamide can be injected i.v. over 3 minutes, which will provoke a maximal rise in growth hormone 10–70 minutes after the nadir of blood sugar (BODEN, 1967); whereas normally the growth hormone concentration will rise from very low or zero values up to supranormal values after 1 hour, in the acromegalic the elevated values are scarcely influenced, which indicates an autonomic or a maximal growth hormone secretion. Sometimes, however, there is an exaggerated increase or a paradoxical decrease, indicating that hypothalamic control is not excluded (CRYER, 1969). An insufficiency can be diagnosed if there is no increase in growth hormone after stimulation, or only an insufficient one. Stimulation can also be realized by an infusion of 0.5 mg arginine per kg body weight i.v. (PARKER, 1967), which is less unpleasant for the patient and does not involve the same risks in pituitary insufficiency. Glucagon also stimulates growth hormone secretion (see p. 90). A glucagon stimulation test has been described (1 mg i.m.); it is thought to be innocuous and reliable in distinguishing between patients deficient in growth hormone and normals (MITCHELL, 1970). Experience will show which of these stimulation tests will be the most useful.

d) Inhibition Test

The glucose inhibition test is usually used for the diagnosis of acromegaly. After a glucose load of 100 g in the normal, the growth hormone decreases during the first hour and increases again after 180 to 240 minutes. The growth hormone of the acromegalic patient is usually hardly affected, if at all (BECK, 1965; ROTH, 1967; EARLL, 1967; BODEN, 1968). However, a decrease or even a paradoxical increase may be observed (CRYER, 1969; EARLL, 1967; LAWRENCE, 1970). As a rule, fasting values of over 8 ng/ml are found in active acromegaly.

e) Nitrogen-Retention Test (see p. 100)

f) Plasma Phosphate

The plasma phosphate is much simpler to measure but a less reliable indicator. Growth

hormone leads to an elevated phosphate concentration of over 3.5 mg% in the plasma due to increased phosphate reabsorption in the renal tubulus. The phosphate can, however, vary between normal and very elevated values in the same person (HAMWI, 1960). Elevated phosphate levels are also found in children during growth, in pregnancy, and in nephropathies, as well as in hypoparathyroidism. Thus, it is only of limited value in evaluation of the growth hormone activity.

g) Sulfation Factor (see p. 88)

h) Hydroxyproline Excretion

The amino acid hydroxyproline is an important constituent of collagen. Hydroxyproline, which is mostly bound in polypeptides, is excreted in the urine. It can be relatively simply estimated after hydrolysis with concentrated hydrochloric acid. The excretion in the 24-hour urine must be estimated after 2 days of a diet containing no meat or gelatine. Normal values are relatively constant, and are between 15 and 50 mg/24 (see also Chap. XIV).

The excretion appears to be proportional to collagen turnover, and probably corresponds to the content of soluble collagen in the tissues. According to the metabolic situation the hydroxyproline excretion reflects collagen formation, or decomposition of the finished collagen, or both.

Hydroxyproline excretion is increased in states where bone is being formed and where bone is being broken down. The excretion is also occasionally increased in rheumatoid arthritis, in scleroderma, dermatomyositis, rheumatic fever, the Marfan syndrome and lathyrism.

Values far above the normal limits are found in growing children, particularly before puberty (PROCKOP, 1967).

Greatly elevated values are found in Paget's disease, hyperparathyroidism, hyperthyroidism, and acromegaly. The excretion is normal after inactivation of the acromegaly. Administration of parathormone, thyroxine, or growth hormone leads to an increase in hydroxyproline excretion.

Highly elevated values have been found in active acromegaly, and estimation of the hydroxyproline excretion provides a valuable criterion in the assessment of the activity of the disease in addition to the direct measurement of growth hormone.

The excretion is not significantly reduced in pituitary insufficiency and is not suitable for the diagnosis of states of endocrine hypofunction.

Increased excretion of unbound hydroxyproline is found in the rare condition hydroxyprolinemia, in which there is congenital deficiency of the enzyme hydroxyproline oxidase (EFRON, 1965).

i) Biopsy of the Ribs

In active acromegaly, biopsy of a rib at the junction of the cartilage and bone shows endochondral osteogenesis in the form of groups of vesicular cartilage cells (SULLIVAN, 1963).

References

Reviews

APOSTOLAKIS, M.: Prolactin. Vitam. and Horm. **26**, 197 (1968).

DAUGHADAY, W.H., KIPNIS, D.M.: The growth-promoting and anti-insulin actions of somatotropin. Recent Progr. Hormone Res. **22**, 49 (1966).

FORSYTH, J.A., EDWARDS, C.R.W.: Human prolactin—its isolation assay and clinical applications. Clin. Endocr. **1**, 293 (1972).

FRANCHIMONT, P.: Sécrétion normale et pathologique de la somatotrophine et des gonadotrophines humaines. Paris: Masson & Cie 1971.

FRIESEN, H., ASTWOOD, E.B.: Hormones of the anterior pituitary body. New Engl. J. Med. **272**, 1216, 1272, 1328 (1965).

GREENWOOD, F.C.: Growth hormone. In: GRAY, C.H., and A.L. BACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

GRUMBACH, M.M.: Growth hormone and growth. Pediatrics **37**, 245 (1966).

HARRIS, G.W., DONOVAN, B.T.: The pituitary gland. London: Butterworths 1966.

ILLIG, R., ZACHMANN, M., PRADER, A.: Menschliches Wachstumshormon. Klin. Wschr. **47**, 117 (1969).

LOCKE, W., SCHALLY, A.V.: The hypothalamus and pituitary in health and disease. Springfield: Thomas 1972.

MASON, A.S.: Human growth hormone. London: Heinemann 1972.

PASTEELS, J.L., ROBYN, C. (eds.): International Symposium on Human Prolactin. Excerpta Medica Congress Series (in print).

PECILE, A., MÜLLER, E.: Growth hormone. Amsterdam: Excerpta med. Found. 1968.

— MÜLLER, E.E.: Growth and growth hormone. Amsterdam: Excerpta medica 1972.

ROOT, A.W.: Human pituitary growth hormone. American Lecture Series. Springfield, Illinois: Thomas 1972.

SIREK, O.V., SIREK, A.: The physiology of growth hormone. Ergebn. inn. Med. Kinderheilk. **21**, 217 (1964).

WEIL, R.: Pituitary growth hormone and intermediary metabolism. Acta endocr. (Stockh.), Suppl. **98** zu Vol. 49 (1965).

WOLSTENHOLME, G.E.W., KNIGHT, J. (eds.): Lactogenic hormones. Edinburgh and London: Churchill Livingstone 1972.

Chemistry and Physiology of Prolactin

BARTKE, A., LLOYD, C.W.: Influence of prolactin and pituitary isografts on spermatogenesis in dwarf mice and hypophysectomized rats. J. Endocr. **46**, 321 (1970).

FRANTZ, A.G., KLEINBERG, D.L., NOEL, G.L.: Studies on prolactin in man. Recent Progr. Hormone Res. **28**, 527 (1972).
FRIESEN, H., HWANG, P.: Human prolactin. Ann. Rev. Med. **24**, 251 (1973).
HORROBIN, D.F., LLOYD, I.J., LIPTON, A., BURSTYN, B.G., DURKIN, N., MUIRURI, K.L.: Actions of prolactin on human renal function. Lancet **1971 II**, 352.
HWANG, P., GUYDA, H., FRIESEN, H.: A radioimmunoassay for human prolactin. Proc. nat. Acad. Sci. (Wash.) **68**, 1902 (1971).
LI, CH.H., DIXON, J.S., LO, T.B., PNAKOV, Y.A., SCHMIDT, K.D.: Amino-acid sequence of ovine lactogenic hormone. Nature (Lond.) **224**, 695 (1969).
MANKU, M.S., HORROBIN, D.F., BURSTYN, P.G.: Prolactin and ADH release. Lancet **1972 I**, 1243.
MARCOVITZ, S., FRIESEN, H.: Regulation of prolactin secretion in man. Clin. Res. **19**, 773 (1971).
MEITES, J., LU, K.H., WUTTKE, W., WELSCH, C.W., NAGASAWA, H., QUADRI, S.K.: Recent studies on functions and control of prolactin secretion in rats. Recent Progr. Hormone Res. **28**, 471 (1972).
NIALL, H.D.: Chemistry of prolactin. Recent Progr. Hormone Res. (in print).
NICOLL, C.S., BERN, H.A.: On the actions of prolactin among the vertebrates. Is there a common denominator? In: WOLSTENHOLME, G.EW., and KNIGHT, J. (eds.), Lactogenic hormones, p. 299. Edinburgh-London: Churchill Livingstone 1972.
PASTEELS, J.L., ROBYN, C. (eds.): International Symposium on Human Prolactin. Excerpta Medica Congress Series (in print).
RAMSEY, D.H., BERN, H.A.: Stimulation by ovine prolactin of fluid transfer in everted sac of rat small intestine. J. Endocr. **53**, 453 (1972).
SHERWOOD, L.M.: Human prolactin. New Engl. J. Med. **284**, 774 (1971).
TURKINGTON, R.W.: Human prolactin. Amer. J. Med. **53**, 389 (1972).
— Ectopic production of prolactin. New Engl. J. Med. **285**, 1455 (1971).
— UNDERWOOD, L.E., VAN WYK, J.J.: Elevated serum prolactin levels after pituitary-stalk section in man. New Engl. J. Med. **285**, 707 (1971).

Chemistry and Immunology of Growth Hormone; Decomposition, Excretion, Plasma Concentration

CAMERON, D.P., BURGER, H.G., CATT, K.J., DOIG, H.: Metabolic clearance rate of radio-iodinated human growth hormone in man. J. clin. Invest. **48**, 1600 (1969).
DIXON, H.B.F.: Chemistry of pituitary hormones. In: The hormones, vol. V, ed. by PINCUS, THIMANN and ASTWOOD. New York-London: Academic Press 1964.
GELLER, J., LOHR, A.: Identification and measurement of growth hormone in extracts of human urine. J. clin. Endocr. **23**, 1107 (1963).
GEMZELL, C.A., LI, C.H.: Estimation of growth hormone content in a single human pituitary. J. clin. Endocr. **18**, 146 (1958).
GLICK, S.M., ROTH, J., YALOW, R.S., BERSON, S.A.: The regulation of growth hormone secretion. Recent Progr. Hormone Res. **21**, 241 (1965).
HONDA, Y., TAKAHASHI, K., TAKAHASHI, S., AZUMI, K., IRIE, M., SAKUMA, M., DSUSHIMA, T., SHIZUME, K.: Growth hormone secretion during nocturnal sleep in normal subjects. J. clin. Endocr. **29**, 20 (1969).
LARON, Z.: Immunological aspects of anterior pituitary hormones with special emphasis on growth hormone. In: Wachstumshormon und Wachstumsstörungen. Das Cushingsyndrom. Ed. by E. KLEIN. Berlin-Heidelberg-New York: Springer 1965.
— PERTZELAN, A., MANNHEIMER, S., GOLDMANN, I., GUTTMANN, S.: Lack of placental transfer of human growth hormone. Acta endocr. (Kbh.) **53**, 687 (1966).
LI, C.H.: The chemistry of human pituitary growth hormone: 1956—1966. In: PECILE and MÜLLER, Growth hormone. Amsterdam: Excerpta med. Found. 1968.
— LIU, W.K.: Human pituitary growth hormone. Experientia (Basel) **20**, 169 (1964).
— — DIXON, J.S.: Human pituitary growth hormone. XII. The aminoacid sequence of the hormone. J. Amer. chem. Soc. **88**, 2050 (1966).
PARKER, M.L., UTIGER, R.D., DAUGHADAY, W.H.: Studies on growth hormone. II. The physiological disposition and metabolic fate of human growth hormone in man. J. clin. Invest. **41**, 262 (1962).
RABEN, M.S.: Human growth hormone. Recent Progr. Hormone Res. **15**, 71 (1959).
ROOS, P., FEVOLD, H.R., GEMZELL, C.H.: Preparation of human growth hormone by gel filtration. Biochim. biophys. Acta (Amst.) **74**, 525 (1963).
SPELLACY, W.N., CARLSON, K.L., SCHADE, S.L.: Human growth hormone levels in normal subjects receiving an oral contraceptive. J. Amer. med. Ass. **202**, 451 (1967).
TAYLOR, A.L.: Metabolic clearance and production rates of human growth hormone. J. clin. Invest. **48**, 2349 (1969).

Substances Related to Growth Hormone

ANSELMINO, K.J., HOFFMANN, F.: Über das Fettstoffwechselhormon des Hypophysenvorderlappens (Lipotropin) und seine Bedeutung für die Fettumsetzung unter physiologischen und pathologischen Bedingungen. Dtsch. med. Wschr. **90**, 1697 (1965).
— — Über den pankreatropen (insulotropen) Wirkstoff des Hypophysenvorderlappens. Dtsch. med. Wschr. **91**, 1401 (1966).
— — Über die parathyreotrope Wirkung des Hypophysen-Vorderlappens. Med. Welt **19**, 1621 (1968).
BECK, P., DAUGHADY, W.H.: Human placental lactogen: Studies of its acute metabolic effects and disposition in normal men. J. clin. Invest. **46**, 103 (1967).
— PARKER, M.L., DAUGHADY, W.H.: Radioimmunologic measurements of human plancental lactogen plasma by a double antibody method during normal and diabetic pregnancies. J. clin. Endocr. **25**, 1457 (1965).
CHALMERS, T.M., PAWAN, G.L.S., KERWICK, A.: Fat-mobilising and ketogenic activity of urine extracts: relation to corticotrophin and growth hormone. Lancet **1960 II**, 6.
FORSYTH, I.A.: Prolactin and placental lactogens. In: GRAY, C.H., and A.L. BACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.
GREENWOOD, F.C., HUNTER, W.M., KLOPPER, A.: Assay of human growth hormone in pregnancy at parturition and in lactation. Detection of a growth hormone-like substance from the placenta. Brit. med. J. **1964 I**, 22.
HUGGINS, A.K., OTTAWAY, J.H.: Purification and properties of a hypoglycaemic peptide from ox growth hormone. J. Endocr. **23**, 193 (1961).
JOSIMOVICH, J.B., MACLAREN, J.A.: Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone. Endocrinology **71**, 209 (1962).
KAPLAN, S.L., GRUMBACH, M.M.: Studies of a human and simian placental hormone with growth hormone-like and prolactin-like activities. J. clin. Endocr. **24**, 80 (1964).
— — Immunoassay for human chorionic "Growth hormone-prolactin" in serum and urine. Science **147**, 751 (1965).
NADLER, A.C., SONENBERG, M., NEW, M.I., FREE, C.A.: Growth hormone activity in man with components of

tryptic digests of bovine growth hormone. Metabolism **16**, 830 (1967).
RUDMAN, D.: The adipokinetic property of hypophyseal peptides. Ergebn. Physiol. **56**, 297 (1965).
— HIRSCH, R. L., KENDALL, F. E., SEIDMAN, F., BROWN, S. J.: An adipokinetic component of the pituitary gland: purification, physical, chemical and biological properties. Recent Progr. Hormone Res. **18**, 89 (1962).
SCHWANDT, P., KARL, H. J., THÜNER, J., KNEDEL, M.: Untersuchungen über eine lipolytisch wirksame Substanz aus Schweinehypophysen (Fraktion H.), Extraktion und Reinigung der lipolytischen Fraktion. Z. ges. exp. Med. **147**, 246 (1968).
SONENBERG, M., DELLACHA, J. M.: Anabolic effects in man of papain digests of bovine growth hormone. J. clin. Endocr. **27**, 1035 (1967).
STEELMAN, S. L., GLITZER, M. S., OSTLIND, D. A., MUELLER, J. F.: Biological properties of the growth hormone-like factor from the plerocercoid of Spirometra mansonides. Recent Progr. Hormone Res. **27**, 97 (1971).
TRYGSTAD, O.: The lipid-mobilizing effect of some pituitary gland preparations. Acta endocr. (Kbh.) **56**, 626 (1967); **57**, 81 (1968).

Sulfation Factor, Somatomedin, NSILA-S

DAUGHADAY, W. H.: Sulfation factor regulation of skeletal growth. Amer. J. Med. **50**, 277 (1971).
FROESCH, E. R.: Discussion to VAN WIJK, J. J. Somatomedin: An insulin-like hormone under growth hormone control. Recent Progr. Hormone Res. (in print).
HALL, K.: Human somatomedin. Determination, occurrence, biological activity and purification. Acta endocr. (Kbh.) **70**, Suppl. 163, 1—52 (1972).
LABHART, A., OELZ, O., BÜNZLI, H. F., HUMBEL, R. E., RITSCHARD, W. J., FROESCH, E. R.: Recent investigation of the nature of nonsuppressible, acid ethanol soluble insulin-like activity (NSILA-S). Israel J. med. Sci. **8**. 901 (1972).
MORELL, B., FROESCH, E. R.: Fibroblasts as an experimental tool in metabolic and hormone studies. I. Growth and glucose metabolism of fibroblasts in culture. Europ. J. clin. Invest. **3**, 112 (1973).
— — Fibroblasts as an experimental tool in metabolic and hormone studies. II. Effects of insulin and nonsuppressible insulin-like activity (NSILA-S) in fibroblasts in culture. Europ. J. clin. Invest. **3**, 119 (1973).
OELZ, O., FROESCH, E. R., BÜNZLI, H. F., HUMBEL, R. E., RITSCHARD, W. J.: Antibody-suppressible and nonsuppressible insulin-like activities. In: Handbook of physiology, section 7: Endocrinology, vol. 1, D. F. STEINER and N. FREINKEL (eds.), p. 685—702. Washington: American Physiological Society 1972.
ZINGG, A., FROESCH, E. R.: NSILA-S as a growth hormone mediated growth factor. Diabetologia (in print).

Effect on Protein Metabolism

CATER, D. B., HOLMES, B. E., MEE, L. K.: The effect of growth hormone upon cell division and nucleic acid synthesis in the regenerating liver of the rat. Biochem. J. **66**, 482 (1957).
DAUGHADAY, W. H., KIPNIS, D. M.: The growth-promoting and anti-insulin actions of somatotropin. Recent Progr. Hormone Res. **22**, 49 (1966).
ENGEL, F. L., KOSTYO, J. L.: Metabolic actions of pituitary hormones. In: The hormones, vol. V., ed. by PINCUS, THIMANN and ASHWOOD. New York-London: Academic Press 1964.
HJALMARSON, A.: Analysis of the biphasic action of growth hormone in vitro on the rat diaphragm. Acta endocr. (Kbh.), Suppl. **126**, to Bd. 57 (1968).
— Sensitivity of the rat diaphragm to growth hormone. Acta endocr. (Kbh.), Suppl. **126**, zu Bd. 57 (1968).
— Temporal relationship of the growth hormone effects on amino acid transport and protein synthesis in isolated rat diaphragm. Acta endocr. (Kbh.), Suppl. **126**, to Bd. 57 (1968).
JEFFERSON, L. S., KORNER, A.: A direct effect of growth hormone on the incorporation of precursors into proteins and nucleic acids of perfused rat liver. Biochem. J. **104**, 826 (1967).
KNOBIL, E., HOTCHKISS, J.: Growth hormone. Ann. Rev. Physiol. **26**, 47 (1964).
KORNER, A.: Growth hormone control of biosynthesis of protein and ribonucleic acid. Recent Progr. Hormone Res. **21**, 205 (1965).
— Anabolic action of growth hormone. Ann. N.Y. Acad. Sci. **148**, 408 (1968).
— Growth hormone control of protein synthesis. In: PECILE and MÜLLER, Growth hormone. Amsterdam: Excerpta med. Found. 1968.
KOSTYO, J. L., KNOBIL, E.: Stimulation of leucine-2-C^{14} incorporation into protein of isolated rat diaphragm by simian growth hormone added in vitro. Endocrinology **65**, 525 (1959).
MOON, H. D., JENTOFT, V. L., LI, C. H.: Effect of human growth hormone on growth of cells in tissue culture. Endocrinology **70**, 31 (1962).
SÖLING, H. D., KNEER, P., DRÄGERT, W., CREUTZFELDT, W.: Die Wirkung von Insulin auf den Stoffwechsel der isolierten perfundierten Leber normaler und alloxandiabetischer Ratten. Teil II. Diabetologia **2**, 32 (1966).
TALWAR, G. P., GUPTA, S. L.: Effect of growth hormone on ribonucleic acid metabolism. Biochem. J. **91**, 565 (1964).

Effect on Carbohydrate and Fat Metabolism

BODEL, P. T., RUBINSTEIN, D., MCGARRY, E. E., BECK, J. C.: Utilization of free fatty acids by diaphragm in vitro. Amer. J. Physiol. **203**, 311 (1962).
FAIN, J. N., KOVACEV, V. P., SCOW, R. O.: Effect of growth hormone and dexamethasone on lypolysis and metabolism in isolated fat cells of the rat. J. biol. Chem. **240**, 3522 (1965).
GOODMAN, H. M.: Early and late effect of growth hormone on the metabolism of glucose in adipose tissue. Endocrinology **76**, 1134 (1965).
HENDERSON, M. J., MORGAN, H. E., PARK, C. R.: Regulation of glucose uptake in muscle. V. The effect of growth hormone on glucose transport in the isolated perfused rat heart. J. biol. Chem. **236**, 2157 (1961).
HJALMARSON, A.: Influence of growth hormone on the sensitivity of rat diaphragm to insulin. Acta endocr. (Kbh.), Suppl. **126**, to Bd. 57 (1968).
IKKOS, D., LUFT, R.: Effects of short term administration of large doses of human growth hormone on carbohydrate metabolism in adult non-diabetic, hypophysectomized women; studies with C^{14} labelled glucose. Acta endocr. (Kbh.) **39**, 567 (1962).
— — GEMZELL, C. A., ALMQUIST, S.: Effect of human growth hormone on glucose tolerance and some intermediary metabolites in man. Acta endocr. (Kbh.) **39**, 547 (1962).
LI, C. H., TANAKA, A., PICKERING, B. T.: Human pituitary growth hormone. VII. in vitro lipolytic activity. Acta endocr. (Kbh.), Suppl. **90**, 155 (1964).
RABEN, M. S.: Growth hormone. New Engl. J. Med. **266**, 31 (1962).
— HOLLENBERG, C. H.: Effect of growth hormone on plasma fatty acids. J. clin. Invest. **38**, 484 (1959).
RABINOWITZ, D., KLASSEN, G. A., ZIERLER, K. I.: Effect of human growth hormone on muscle and adipose tissue metabolism in the forearm of man. J. clin. Invest. **44**, 51 (1965).

RANDLE, P. J., GARLAND, P. B., HALES, C. H., NEWSHOLME, E. A.: The glucose fatty acid cycle and diabetes mellitus. Ciba Found. Coll. Endocr. **15**, 192 (1964).
SWISLOCKI, N. I., SZEGO, C. M.: Acute reduction of plasma nonesterified fatty acid by growth hormone in hypophysectomized and houssay rats. Endocrinology **76**, 665 (1965).
WINEGRAD, A. I., SHAW, W. N., LUKENS, P. D. W., STADIE, W. C., RENOLD, A. E.: Effects of growth hormone in vitro on the metabolism of glucose in rat adipose tissue. J. biol. Chem. **234**, 1922 (1959).
ZAHND, G. R., STEINKE, J., RENOLD, A. E.: Early metabolic effects of human growth hormone. Proc. Soc. exp. Biol. (N. Y.) **105**, 455 (1960).

Effect on General Metabolism and Relation to Diabetes Mellitus

BERGENSTALL, D. M., LIPSETT, M. B.: Metabolic effects of human growth hormone and growth hormone of other species in man. J. clin. Endocr. **20**, 1427 (1960).
BERT, P.: Présence du sucre dans l'urine après accouchement chez une chèvre privée des mamelles. C. R. Soc. biol. (Paris) **7**, 193 (1883).
CAHILL, G. F., JR., HERRERA, M. G., MORGAN, A. P., SOELDNER, J. S., STEINKE, J., LEVY, P. L., REICHARD, G. A., JR., KIPNIS, D. M.: Hormone-fuel interrelationships during fasting. J. clin. Invest. **45**, 1751 (1966).
LEVINE, R., LUFT, R.: The relation between the growth and diabetogenic effects of the so-called growth hormone of the anterior pituitary. Diabetes **13**, 651 (1964).
LUFT, R.: The so-called growth hormone of the anterior pituitary. Acta med. scand., Suppl. **445** to 179 (1966).
— CERASI, E.: Humanes Wachstumshormon und Diabetes. In: Wachstumshormon und Wachstumsstörungen. Das Cushing-Syndrom. Ed. by E. KLEIN. Berlin-Heidelberg-New York: Springer 1965.
— — HAMBERGER, C. A.: Studies on the pathogenesis of Diabetes in acromegaly. Acta endocr. (Kbh.) **50**, 607 (1967).
MITCHELL, M. L., RABEN, M. S., ERNESTI, M.: Use of growth hormone as a diabetic stimulus in man. Diabetes **19**, 196 (1970).
POWELL, E. D. U., FRANTZ, A. G., RABKIN, M. T., FIELD, R. A.: Growth hormone in relation to diabetic retinopathy. New Engl. J. Med. **275**, 922 (1966).
RABEN, M. S.: Growth hormone. Diabetes **14**, 374 (1965).
RABINOWITZ, D., MERIMEE, T. J., BURGESS, J. A.: Growth hormone-insulin interaction. Diabetes **15**, 905 (1966).
RUSSEL, J. A.: Effects of growth hormone on protein and carbohydrate metabolism. Amer. J. clin. Nutr. **5**, 404 (1957).
SCOW, R. O., CHERNICK, S. S.: Hormonal control of protein and fat metabolism in the pancreatectomized rat. Recent Progr. Hormone Res. **16**, 497 (1960).
ZIERLER, K. L., RABINOWITZ, D.: Roles of insulin and growth hormone, based on studies of forearm metabolism in man. Medicine (Baltimore) **42**, 385 (1963).

Effect on Electrolyte and Water Balance

BIGLIERI, E. G., WATLINGTON, CH. O., FORSHAM, P. H.: Sodium retention with human growth hormone and its subfractions. J. clin. Endocr. **21**, 361 (1961).
CORVILAIN, J., ABRAMOW, M.: Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man. J. clin. Invest. **41**, 1230 (1962).
FRASER, R., HARRISON, M.: The effect of growth hormone on urinary calcium excretion. Ciba Found. Coll. Endocr. **13**, 135 (1960).

Effect on Other Endocrine Glands

BIGLIERI, E. G., WATLINGTON, CH. O., FORSHAM, P. H.: Sodium retention with human growth hormone and its subfractions. J. clin. Endocr. **21**, 361 (1961).
FINKELSTEIN, J. W., KOWARSKI, A., SPAULDING, J. S., MIGEON, C. J.: Effect of various preparations of human growth hormone on aldosterone secretion rate of hypopituitary dwarfs. Amer. J. Med. **38**, 517 (1965).
FRASER, R., HARRISON, M.: The effect of growth hormone on urinary calcium excretion. Ciba Found. Coll. Endocr. **13**, 135 (1960).
ROSS, E. J., VAN HOFF, CRABBÉ, W. J., THORN, G. W.: Aldosterone excretion in hypopituitarism and after hypophysectomy in man. Amer. J. Med. **28**, 229 (1960).
ZAHND, G. R., STEINKE, J., RENOLD, A. E.: Early metabolic effects of human growth hormone. Proc. Soc. exp. Biol. (N. Y.) **105**, 455 (1960).

Effect on the Kidney

ASTARABADI, T.: The effect of growth and lactogenic hormones on renal compensatory hypertrophy in hypophysectomized rats. Quart. J. exp. Physiol. **48**, 85 (1963).
CORVILAIN, J., ABRAMOW, M.: Some effects of human growth hormone on renal hemodynamics and on tubular phosphate transport in man. J. clin. Invest. **41**, 1230 (1962).

Effect of Prolactin and Growth hormone on Tumor Growth

BENJAMIN, F., CARPER, D. J., SHERMAN, L., KOLODNY, H. D.: Growth hormone secretion in patients with endometrial carcinoma. New Engl. J. Med. **281**, 1448 (1969).
BOYNS, A. R., GRIFFITHS, K. (eds.). Prolactin and carcinogenesis. Cardiff: Alpha Omega Alpha publishing college buildings 1972.
HENNEMAN, P. H.: Discussion to RABEN, M. S., Human growth hormone. Recent Progr. Hormone Res. **15**, 109 (1959).
MIRAUD, E. A., HOFFMAN, J. G.: Effect of pituitary growth hormone on transplantable mouse tumors. Proc. Soc. exp. Biol. (N. Y.) **95**, 819 (1957).
MUSTACCHI, P., SKIMKIN, M. B.: Occurrence of cancer in acromegaly and in hypopituitarism. Cancer (Philad.) **10**, 100 (1957).
NOBLE, R. L.: Tumors and hormones. In: The Hormones, vol. V, ed. by PINCUS, THIMANN and ASTWOOD. New York-London: Academic Press 1964.
REID, E.: Growth hormone and adrenocortical hormones in relation to experimental tumors: A review. Cancer Res. **14**, 249 (1954).
SALIH, H., FLAX, H., BRANDER, W., HOBBS, J. R.: Prolactin dependence in human breast cancers. Lancer **1972II**, 1103.

Regulation of Growth Hormone Secretion and Interactions between Growth Hormone and Other Hormones

ABRAMS, R. L., PARKER, M. L., BLANCO, S., REICHLIN, S., DAUGHADAY, W. H.: Hypothalamic regulation of growth hormone secretion. Endocrinology **78**, 605 (1966).
BLACKARD, G., HEIDINGSFELDER, S. A.: Adrenergic receptor control mechanism for growth hormone secretion. J. clin. Invest. **47**, 1407 (1968).
DEUBEN, R. R., MEITES, J.: Stimulation of pituitary growth hormone release by a hypothalamic extract in vitro. Endocrinology **74**, 408 (1964).

FAJANS, S.A., FLOYD, J.C., JR., KNOPF, R.F., CONN, J.W.: Effect of amino acids and proteins on insulin secretion in men. Recent Progr. Hormone Res. **24**, 617 (1967).

FRANTZ, A.G., RABKIN, M.T.: Human growth hormone. Clinical measurement, response to hypoglycemia and suppression by corticosteroids. New Engl. J. Med **271**, 1375 (1964).

— — Effects of estrogen and sex difference on secretion of human growth hormone. J. clin. Endocr. **25**, 1470 (1965).

GAGLIARDINO, J.J., BAILEY, J.D., MARTIN, J.M.: Effect of vasopressin on serum levels of human growth hormone. Lancet **1967 I**, 1357.

— MARTIN, J.M.: Stimulation of growth hormone secretion in monkeys by adrenalin, pitressin and adenosine-3'5'-cyclic monophosphoric acid (3'5'-AMP). Acta endocr. (Kbh.) **59**, 390 (1968).

GLICK, S.M., GOLDSMITH, S.: The physiology of growth hormone secretion. In: PECILE and MÜLLER, Growth hormone. Amsterdam: Excerpta med. Found. 1968.

— ROTH, J., YALOW, R.S., BERSON, S.A.: The regulation of growth hormone secretion. Recent Progr. Hormone Res. **21**, 241 (1965).

GREENWOOD, F.C., LANDON, J.: Growth hormone secretion in response to stress. Nature (Lond.) **210**, 540 (1966).

GWINUP, G., STEINBERG, T., KING, C.G., VERNICOS-DANELLIS, J.: Vasopressin-induced ACTH secretion in man. J. clin. Endocr. **27**, 927 (1967).

HUNTER, W.M., FOUSEKA, C.C., PASSMORE, R.: Growth hormone: important role of muscular exercise in adults. Science **150**, 1051 (1965).

— GREENWOOD, F.C.: Studies on the secretion of human-pituitary-growth hormone. Brit. med. J. **1964 I**, 804.

ILLIG, R., PRADER, A.: Effect of testosterone on growth hormone secretion in patients with anorchia and delayed puberty. J. clin. Endocr. **30**, 615 (1970).

IMURA, H., KATO, Y., IKEDA, M., MORIMOTO, M., YAVATA, M., FUKASE, M.: Increased plasma levels of growth hormone during infusion of propanolone. J. clin. Endocr. **28**, 1079 (1968).

IWATSUBO, H., OMORI, K., OKADA, Y., FUKUCHI, M., MIYAI, K., ABE, H., KUMAHARA, Y.: Human growth hormone secretion in primary hypothyroidism before and after treatment. J. clin. Endocr. **27**, 1751 (1967).

KATZ, H.P., YOULTON, R., KAPLAN, S.L., GRUMBACH, M.M.: Growth and growth hormone. III. Growth hormone release in children with primary hypothyroidism and thyrotoxicosis. J. clin. Endocr. **29**, 346 (1969).

KNOPF, R.F., CONN, J.W., FAJANS, S.S., FLOYD, J.C., GUNTSCHE, E.M., RULL, J.A.: Plasma growth hormone response to intravenous administration of amino acids. J. clin. Endocr. **25**, 1140 (1965).

KOWARSKI, A., THOMPSON, R.G., MIGEON, C.J., BLIZZARD, R.M.: Determination of integrated plasma concentrations and true secretion rates of human growth hormone. J. clin. Endocr. **32**, 356 (1971).

LUFT, R., CERASI, E., MADISON, L.L., EULER, U.S.v., DELLA CASA, L., ROOVETE, A.: Effect of a small decrease in blood-glucose on plasma-growth-hormone and urinary excretion of catecholamines in man. Lancet **1966 II**, 154.

MARTIN, L.G., CLARK, J.W., CONNOR, T.B.: Growth hormone secretion enhanced by androgens. J. clin. Endocr. **28**, 425 (1968).

MCCANN, S.M., PORTER, J.C.: Hypothalamic pituitary stimulating and inhibiting hormones: Physiol. Rev. **40**, 240 (1969).

MEITES, J., FIEL, N.J.: Effect of starvation on hypothalamic content of "somatotropin releasing factor" and pituitary growth hormone content. Endocrinology **77**, 455 (1965).

MERIMEE, T.J., BURGESS, J.A., RABINOWITZ, D.: Sex determined variation in serum insulin and growth hormone response to amino acid stimulation. J. clin. Endocr. **26**, 791 (1966).

— RABINOWITZ, D., RIGGS, L., BURGESS, J.A., RIMOIN, D.L., MCKUSICK, V.A.: Plasma growth hormone after Arginine infusion. New Engl. J. Med. **276**, 434 (1967).

MITCHELL, M.L., BYRNE, M.J., SILVER, J.: Growth hormone release by glucagon. Lancet **1969 I**, 289.

PECILE, A., MÜLLER, E.: Suppressive action of corticosteroids on the secretion of growth hormone. J. Endocr. **36**, 401 (1966).

QUABBE, H.J., SCHILLING, E., HELGE, H.: Pattern of growth hormone secretion during a 24-hour fast in normal adults. J. clin. Endocr. **26**, 1173 (1966).

RABINOWITZ, D., MERIMEE, T.J., BURGESS, J.A., RIGGS, L.: Growth hormone and insulin release after arginine: indifference to hyperglycemia and epinephrine. J. clin. Endocr. **26**, 1170 (1966).

REICHLIN, S.: Regulation of somatotrophic hormone secretion. In: HARRIS and DONOVAN, The pituitary gland. London: Butterworths 1966.

— Functions of the median-eminence gland. New Engl. J. Med. **275**, 600 (1966).

ROTH, J., GLICK, S.M., CUATRECASAS, P., HOLLANDER, CH.S.: Acromegaly and other disorders of growth hormone secretion. Ann. intern. Med. **66**, 760 (1967).

SCHALLY, A.V., ARIMURA, A., BOWERS, C.Y., KASTIN, A.J., SAWANO, S., REDDING, T.W.: Hypothalamic neurohormones regulating anterior pituitary function. Recent Progr. Hormone Res. **24**, 497 (1968).

— KASTIN, A.J., LOCKE, W., BOWERS, C.V.: Hypothalamic releasing and inhibiting factors. In: GRAY, C.H., and A.L. BACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

— SAWANO, S., MÜLLER, E.E., ARIMURA, A., BOWERS, C.Y., REDDING, T.W., STEELMAN, S.L.: Hypothalamic growth hormone-releasing hormone (GRH). Purification and in vivo and in vitro studies. In: PECILE and MÜLLER, Growth hormone. Amsterdam: Excerpta med. Found. 1968.

SOYKA, L.F., CRAWFORD, J.D.: Antagonism by cortisone of the linear growth induced in hypopituitary patients and hypophysectomized rats by human growth hormone. J. clin. Endocr. **25**, 469 (1965).

STIEL, J.N., ISLAND, D.P., LIDDLE, G.W.: Effect of glucocorticoids on plasma growth hormone in man. Metabolism **19**, 158 (1970).

UNGER, R.H., EISENTRAUT, A.M., MADISON, L.L., SIPERSTEIN, M.D.: Fasting levels of growth hormone in men and women. Nature (Lond.) **205**, 804 (1965).

VINIK, A., PIMSTONE, B., BUCHANAN-LEE, B.: Impairment of hyperglycemia-induced growth hormone suppression in hyperthyroidism. J. clin. Endocr. **28**, 1534 (1968).

WEBER, B., HELGE, H., QUABBE, H.-J.: Glucagon-induced growth hormone release in children. Acta endocr. (Kbh.) **65**, 323 (1970).

ZACHMANN, M., PRADER, A.: Interactions of growth hormone with other hormones. In: (A. STUART MASON, ed.) Human growth hormone. London: Heinemann 1972.

ZAHND, G.R., NADEAU, A., VON MÜHLENDAHL, K.E.: Effect of corticotrophin on plasma levels of human growth hormone. Lancet **1969 II**, 1278.

Etiology, Pathogenesis, Pathologic Anatomy and Clinical Aspects of Pituitary Insufficiency

AGUILÓ, F., VEGA, L.A., HADDOCK, L., RODRIGUEZ, O.: Diabetes insipidussyndrome in hypopituitarism of pregnancy. Acta endocr. (Kbh.), **Suppl.** 137 zu Vol. 60, 1969.

ARSDEL, P.P. VAN, JR., WILLIAMS, R.H.: SIMOND's disease. Evaluation of certain laboratory tests used in diagnosis. Amer. J. Med. **20**, 4 (1956).

AUBRY, R.H., NANKIN, H.R., MOSES, A.M., STREETEN, D.H.P.: Measurement of the osmotic threshold for

vasopressin release in human subjects and its modification by cortisol. J. clin. Endocr. **25**, 1481 (1965).
BETHUNE, J. E., NELSON, D. H.: Hyponatremia in hypopituitarism. New Engl. J. Med. **272**, 771 (1965).
BLEULER, M.: Endokrine Psychiatrie. Aus: Psychiatrie der Gegenwart, Bd. I/13. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
BORER, F., MACH, R. S.: L'insuffisance hypophysaire de l'adulte. Praxis **52**, 1462, 1506 (1963).
BRENNAN, C. F. R., MALONE, G. S., WEAVER, J. A.: Pituitary necrosis in diabetes mellitus. Lancet **1956II**, 12.
BRISMAN, R., HUGHES, J. E. O., HOLUB, D. A.: Endocrine function in nineteen patients with empty sella syndrome. J. clin. Endocr. **34**, 570 (1972).
BRUNNER, H. E., LABHART, A.: Das Koma bei Hypophyseninsuffizienz. Differentialdiagnose und Behandlung. Internist (Berl.) **6**, 406 (1965).
CAPLAN, R. H., DOBBEN, G. D.: Endocrine studies in patients with "empty sella syndrome". Arch. intern. Med. **123**, 611 (1969).
Clinico-pathological Conference: Chromophobe adenoma of the pituitary gland with hyponatremia. New Engl. J. Med. **270**, 143 (1964).
EGLOFF, B., FISCHBACHER, W., GOUMOËNS, E. v.: Lymphomatöse Hypophysitis mit Hypophyseninsuffizienz. Schweiz. med. Wschr. **99**, 1499 (1969).
ENGSTRÖM, W. W.: Reappearance of menstruation and gonadotropins with treatment of secondary and adrenocortical failure in SHEEHAN's syndrome. J. clin. Endocr. **21**, 1007 (1961).
EPSTEIN, S., PIMSTONE, B. L., VILLIERS, J. C. DE, JACKSON, W. P. U.: Pituitary apoplexy in five patients with pituitary tumors. Brit. med. J. **1971 II**, 267.
ESCAMILLA, R. F., LISSER, H.: Simmonds disease. A clinical study with review of the literature. Differentiation from anorexia nervosa by statistical analysis of 595 cases, 101 of which were proved pathologically. J. clin. Endocr. **2**, 65 (1942).
EVANS, H. W.: SHEEHAN's Syndrome with diabetes insipidus. Amer. J. Med. **28**, 648 (1960).
FALKHEDEN, T.: Renal function following hypophysectomy in man. Acta endocr. (Kbh.) **42**, 571 (1963).
— WICKBOM, J.: Renal function and kidney size following hypophysectomy in man. Acta endocr. (Kbh.) **48**, 348 (1965).
FARQUHARSON, R. F.: Simmonds disease. Springfield: Thomas 1950.
GASTINEAU, C. F., FRETHEM, A. A., SVIEN, H. J., MAGID, G. A., KEARNS, TH. P.: Hyponatremia after section of pituitary stalk for diabetic retinopathy. Proc. Mayo Clin. **42**, 400 (1967).
GOUDIE, R. B., PINKERTON, P. H.: Anterior hypophysitis and Hashimoto's disease in a young woman. J. Path. Bact. **83**, 584 (1962).
HUME, R., ROBERTS, G. H.: Hypophysitis and Hypopituitarism: Report of a case. Brit. med. J. **1967 II**, 548.
KAPPELER, R.: Hyperthyreose und Panhypopituitarismus im Verlauf eines chromophoben Adenoms der Hypophyse. Schweiz. med. Wschr. **89**, 367 (1959).
KAUFMAN, B.: "Empty" sella turcica: Manifestation of the intrasellar subarachnoid space. Radiology **90**, 931 (1968).
KERKHOVEN, P.: Hypophysenvorderlappennekrose bei Verbrennungsschock. Schweiz. med. Wschr. **85**, 1066 (1965).
KIND, H.: Die Psychiatrie der Hypophyseninsuffizienz speziell der Simmondsschen Krankheit. Fortschr. Neurol. Psychiat. **26**, 501 (1958).
KOVACS, K., NAGY, E., MAROSI, G.: Beeinflussung der durch Hexadimethrin-bromid verursachten Gewebsveränderungen bei Ratten mit Hypophysenhinterlappenextrakt bzw. Diaethylstilben. Endokrinologie **52**, 276 (1967).
LAWRENCE, A. M., GOLDFINE, I. D., KIRSTENIG, L.: Growth hormone dynamics in acromegaly. J. clin. Endocr. **31**, 239 (1970).
LINDQVIST, G.: Mental changes after transsphenoidal hypophysectomy. Acta psychiatr. scand., Suppl. **190**, zu Bd. 42 (1966).
LUFT, R., OLIVECRONA, H., EULER, U. v., IKKOS, D., LJUNGGREN, H., NILSON, L. B., SEKKENES, J., SJÖGREN, B., WASCHEWSKY, H. J.: Die endokrinen Insuffizienzen nach der Hypophysektomie beim Menschen. Helv. med. Acta **22**, 338 (1955).
MARTIN, J. E., MACDONALD, P. C., KAPLAN, N. M.: Successful pregnancy in a patient with Sheehan's Syndrome. New Engl. J. Med. **282**, 425 (1970).
MEADOR, C. K., WORRELL, J. L.: The sella turcica in postpartum pituitary necrosis (SHEEHAN's Syndrome). Ann. intern. Med. **65**, 259 (1966).
MUKHTAR, E., WILKINSON, R., ALEXANDER, L., APPLETON, D., HALL, R.: Thyroid function in acromegaly. Lancet **1971 II**, 279.
OLSON, D. R., GUIOT, G., DEROME, P.: The symptomatic empty sella. J. Neurosurg. **37**, 533 (1972).
PLAUT, A.: Pituitary necrosis in routine necropsies. Amer J. Path. **28**, 883 (1952).
PURNELL, D. C., RANDALL, R. V., RYNEARSON, E. H.: Post partum pituitary insufficiency (SHEEHAN's Syndrome). Review of 18 cases. Proc. Mayo Clin. **39**, 321 (1964).
RABKIN, M. T., FRANTZ, A. G.: Hypopituitarism: a study of growth hormone and other endocrine functions. Ann. intern. Med. **64**, 1197 (1966).
RANDALL, R. E., JR., SPONG, F. W.: Calcification of auricular cartilage in a patient with hypopituitarism. New Engl. J. Med. **269**, 1135 (1963).
RIGGS, B. L., RANDALL, R. V., WAHNER, H. W., JOWSEY, J., KELLY, P. J., SINGH, M.: The nature of the metabolic bone disorder in acromegaly. J. clin. Endocr. **34**, 911 (1972).
ROSS, E. J., VAN'T HOFF, N., CRABBÉ, J., THORN, G. W.: Aldosterone excretion in hypopituitarism and after hypophysectomy in man. Amer. J. Med. **28**, 229 (1960).
SCHALCH, D. S., BURDAY, S. Z.: Antepartum pituitary insufficiency in diabetes mellitus. Ann. intern. Med. **74**, 357 (1971).
SCHNEEBERG, N. G., PERLOFF, W. H., ISRAEL, S. L.: Incidence of unsuspected Sheehans syndrome. J. Amer. med. Ass. **172**, 20 (1960).
SCHÜPBACH, A.: Postpartuales Myxödem und Simmondssche Krankheit. Schweiz. med. Wschr. **81**, 610 (1951).
SHEEHAN, H. L.: Incidence of post partum hypopituitarism. Amer. J. Obst. & Gynec. **68**, 202 (1954).
— The repair of post-partum necrosis of the anterior lobe of the pituitary gland. Acta endocr. (Kbh.) **48**, 40 (1965).
— DAVIS, J. C.: Pituitary necrosis. Brit. med. Bull. **24**, 59 (1968).
— STANFIELD, J. P.: The pathogenesis of post partum necrosis of the anterior lobe of the pituitary gland. Acta endocr. (Kbh.) **37**, 479 (1961).
— SUMMERS, V. K.: The syndrome of hypopituitarism. Quart. J. Med., N. S. **18**, 319 (1949).
— WHITEHEAD, R.: The neurohypophysis in post-partum hypopituitarism. J. Path. Bact. **85**, 145 (1963).
SIMMONDS, M.: Über Hypophysisschwund mit tödlichem Ausgang. Dtsch. med. Wschr. **7**, 322 (1914).
WIESENDANGER, M.: Über die Häufigkeit der postpartualen Hypophysenvorderlappeninsuffizienz (Sheehan-Syndrom). Diss. Zürich 1959.

Partial Hypophyseal Insufficiency

DAYER-HAENNI, A.: Les insuffisances hypophysaires partielles. Praxis **48**, 333, 361 (1959).
GOLDMAN, J. K., CAHILL, G. F., JR., THORN, G. W.: Gigantism with hypopituitarism. Amer. J. Med. **34**, 407 (1963).
MADDOCK, W. O., LEACH, R. B., MYERS, G. B.: Basophile failure, a syndrome characterized by failure of ACTH and gonadotropin secretion associated with intact thyreotropin secretion. J. Lab. clin. Med. **38**, 924 (1951).

OBERDISSE, K.: Die partielle Vorderlappeninsuffizienz. 4. Sympos. der Dtsch. Ges. für Endocrinol. Berlin: Springer 1957.

ODELL, W. D.: Isolated deficiencies of anterior pituitary hormones. J. Amer. med. Ass. **197**, 1006 (1966).

— GREEN, G. M., WILLIAMS, R. H.: Hypoadrenotropism: the isolated deficiency of adrenotropic hormone. J. clin. Endocr. **20**, 1017 (1960).

PERKOFF, G. T., EIK-NES, K., CARNES, N. W., TYLER, F. H.: Selective hypopituitarism with deficiency of anterior pituitary basophils: a case report. J. clin. Endocr. **20**, 1269 (1960).

SARVER, M. E., SABEH, G., FETTERMAN, G. H., WALD, N., DANOWSKI, T. S.: Fractional hypopituitarism with giantism and normal sella turcica. New Engl. J. Med. **271**, 1286 (1964).

Special Forms of Anterior, Pituitary Insufficiency

BAUER, H. C.: Endocrine and other clinical manifestations of hypothalamic disease. A survey of 60 cases with autopsies. J. clin. Endocr. **14**, 13 (1954).

HEDINGER, CHR., HÜRZELER, D.: Hypopituitarismus bei Dystopie des Hypophysenhinterlappens. Acta endocr. (Kbh.) **14**, 170 (1953).

HUME, R., ROBERTS, G. H.: Hypophysitis and Hypopituitarism: report of a case. Brit. med. J. **1967 I**, 548.

KRAYENBÜHL, H., ZOLLINGER, H. U.: Malignes metastasierendes Pinealocytom mit dem klinischen Bild der Dystrophia adiposo-genitalis. Schweiz. Arch. Neurol. **51**, 77 (1943).

LUNDBERG, P. O., GEMZELL, C.: Dysplasia of the sella turcica: clinical and laboratory investigations in three cases. Acta endocr. (Kbh.) **52**, 478 (1966).

NOWAKOWSKI, H.: Über die endokrine Symptomatik bei Erkrankungen des Hypothalamus. Verh. dtsch. Ges. inn. Med. **61**, 49 (1955).

PRIESEL, A.: Über die Dystopie der Neurohypophyse. Virchows Arch. path. Anat. **266**, 407 (1927).

Pituitary Dwarfiom

BRASEL, J. A., WRIGHT, J. C., WILKINS, L., BLIZZARD, R. M.: An evaluation of seventy-five patients with hypopituitarism beginning in childhood. Amer. J. Med. **38**, 484 (1965).

DAUGHADAY, W. H., PARKER, M. L.: Human pituitary growth hormone. Ann. Rev. Med. **16**, 47 (1965).

GOODMAN, H. G., GRUMBACH, M. M., KAPLAN, S. L.: Isolated growth-hormone and multiple pituitary-hormone deficiencies. New Engl. J. Med. **278**, 57 (1968).

GRUMBACH, M. M.: Growth hormone and growth. Pediatrics **37**, 245 (1966).

HENNEMAN, P. H.: The effect of human growth hormone on growth of patients with hypopituitarism. J. Amer. med. Ass. **205**, 828 (1968).

HUBBLE, D.: Diagnosis of hypopituitarism in childhood. Arch. Dis. Childh. **42**, 228 (1967).

ILLIG, R., ZACHMANN, M., PRADER, A.: Menschliches Wachstumshormon. Klin. Wschr. **47**, 117 (1969).

KRAYENBÜHL, H., PRADER, A.: Traitement endocrinien des troubles de la croissance chez des malades opérés d'un craniophyryngiome. Neuro-chirurgie **8**, 223 (1962).

LARON, Z., PERTZELAN, A., KARP, M.: Pituitary dwarfism with high serum levels of growth hormone. Israel J. med. Sci. **4**, 883 (1968).

NAJJAR, S., BLIZZARD, R. M.: Current concepts regarding human growth hormone (Somatotropin). Pediat. Clin. N. Amer. **13**, 437 (1966).

ODELL, W. D.: Isolated deficiencies of anterior pituitary hormones. Symptoms and diagnosis. J. Amer. med. Ass. **197**, 1006 (1966).

PECILE, A., MÜLLER, E. E. (ed.): Growth hormone. Proc. 1st Int. Symp., Milan Sept. 11—13, 1967. Excerpta Medica Foundation, Internat. Congr. Ser. No 158 (1968).

PFEIFFER, E. F., MELANI, F.: Menschliches Wachstumshormon. Dtsch. med. Wschr. **93**, 846 (1968).

POWELL, G. F., BRASEL, J. A., BLIZZARD, R. M.: Emotional deprivation and growth retardation simulating idiopathic hypopituitarism I. New Engl. J. Med. **276**, 1271 (1967).

PRADER, A., ZACHMANN, M., POLEY, J. R., ILLIG, R.: The metabolic effect of a small uniform dose of human growth hormone in hypopituitary dwarfs and in control children. I. Nitrogen, α-amino-N, creatine-creatinine and calcium excretion and serum ureá-N, α-amino-N, inorganic phosphorus and alkaline phosphatase. Acta endocr. (Kbh.) **57**, 115 (1968).

— — — — SZÉKY, J.: Long-term treatment with human growth hormone (Raben) in small doses. Evaluation of 18 hypopituitary patients. Helv. paediat. Acta **22**, 423 (1967).

RIMOIN, D. L., MERIMÉE, T. J., MCKUSICK, V. A.: Growth hormone deficiency in man: An isolated, recessively inherited defect. Science **152**, 1635 (1966).

SHEIKHOLISLAM, B., STEMPFEL, R. S.: Familial growth hormone (GH) deficiency. Ped. Res. **1**, 205 (1967).

Functional Hypopituitarism

DECOURT, J., MICHARD, J. P.: Le retentissement endocrinien des maigreurs névropathiques. Sem. Hôp. Paris **34**, 2139 (1958).

GALLETTI, P. M., LABHART, A.: Etude de la fonction thyroidienne dans l'anorexie mentale. Helvet. med. Acta **22**, 536 (1955).

JORES, A.: Die Anorexia nervosa als endokrinologisches Problem. Acta endocr. (Kbh.) **17**, 206 (1951).

PERLOFF, W. H., LASCHÉ, E. M., NODINE, J. H., SCHNEEBERG, N. G., VIEILLARD, C. B.: The starvation state and functional hypopituitarism. J. Amer. med. Ass. **155**, 1307 (1954).

SIEBENMANN, R. E.: Zur pathologischen Anatomie der Anorexie nervosa. Schweiz. med. Wschr. **1955**, 530.

UEHLINGER, E.: Pathologische Anatomie der Hungerkrankheit und des Hungerödems. In: HOTTINGER, GSELL, UEHLINGER, SALZMANN u. LABHART, Hungerkrankheit, Hungerödem, Hungertuberkulose. Basel: Benno Schwabe & Co. 1948.

WISMER, R.: Les fonctions endocriniennes dans l'anorexie mentale. Praxis **54**, 981 (1965).

Overproduction of Prolactin (Syndrome of Galactorrhea and Amenorrhea)

APOSTOLAKIS, M.: Prolactin. Vitam. and Horm. **26**, 197 (1968).

ARGONZ, J., CASTILLO, E. B. DEL: A syndrome characterized by estrogenic insufficiency, galactorrhea and decreased urinary gonadotropin. J. clin. Endocr. **13**, 79 (1953).

BECK, J. C.: Ciba Symposium on lactogenic hormones. Edinburgh and London: Churchill Livingstone 1972.

BERN, H. A.: Hormones and endocrine glands of fishes: Studies of fish endocrinology reveal major physiologic and evolutionary problems. Science **158**, 455 (1967).

BESSER, G. M., PARKE, L., EDWARDS, C. R. W., FORSYTH, I. A., MCNEILLY, A. S.: Galactorrhea: Successful treatment with reduction of plasma prolactin levels by brom-ergocryptine. Brit. med. J. **1972 3**, 669.

BRUN DEL RE, R., DEL POZO, E., DE GRANDI, P., FRIESEN, H. G., HINSELMANN, M.: Prolactin inhibition and suppression of puerperal lactation by a Br-ergocryptine

(CB 154): a comparison with estrogen. Obestet Gynec (in print).
CAUFIELD, C.J., BATES, R.W.: Nonpuerperal galactorrhea. New Engl. J. Med. **273**, 897 (1965).
Clinico-pathological Conference: Non puerperal galactorrhoea, amernorrhoea and visual loss. Amer. J. Med. **33**, 591 (1962).
DEL POZO, E., BRUN DEL RE, R., VARGA, L., FRIESEN, H.G.: The inhibition of prolactin secretion in man by CB 154 (2-Br-α-ergocryptine). J. clin. Endocr. **35**, 768 (1972).
— FRIESEN, H., BURMEISTER, P.: Endocrine profile of a specific prolactin inhibitor: Br-ergocryptine (CB 154). Schweiz. med. Wschr. **103**, 847 (1973).
FLÜCKIGER, E.: Drugs and the control of prolactin secretion. In: Prolactin and Carcinogenesis (BOYNS, A.R., GRIFFITHS, K., eds.), Proc. 4th Tenovus Workshop, Cardiff, Cardiff: Alpha Omega Alpha publishing college buildings 1972.
— WAGNER, H.E.: 2-Br-alpha-Ergokryptin: Beeinflussung von Fertilität und Laktation bei der Ratte. Experientia (Basel) **24**, 1130 (1968).
FORBES, A.P., HENNEMAN, P.H., GRISWOLD, G.C., ALBRIGHT, F.: Syndrome characterized by galactorrhea, amenorrhea and low urinary FSH: comparison with acromegaly and normal lactation. J. clin. Endocr. **14**, 265 (1954).
FRANTZ, A.G., KLEINBERG, D.L., NOEL, G.L.: Studies on prolactin in man. Recent Progr. Hormone Res. **28**, 573 (1972).
FRIESEN, H., WEBSTER, B.R., HWANG, P., GUYDA, H., MUNRO, R.E., READ, L.: Prolactin synthesis and secretion in a patient with the Forbes Albright syndrome. J. clin. Endocr. **34**, 192 (1972).
GUINET, P., PUTELAT, R., TOMMASI, M., DESCOURS, C., FRANCHET, G.: Un cas de craniopharyngéome avec galactorrhée et aménorrhée. Ann. Endocr. (Paris) **22**, 385 (1961).
NIALL, H.D.: Chemistry of prolactin. Recent Progr. Hormone Res. (in press) (19).
RACADOT, J., VILA-PORCILE, E., PEILLON, F., OLIVIER, L.: Adénomes hypophysaires à cellules à prolactine: étude structurale et ultrastructurale, corrélations anatomocliniques. Ann. Endocr. (Paris) **32**, 298 (1971).
SHERWOOD, L.M.: Human Prolactin. New Engl. J. Med. **284**, 774 (1971).
TURKINGTON, R.W.: Human prolactin. An ancient molecule proves new insights for clinical medicine. Amer. J. Med. **53**, 389 (1972).
— Inhibition of prolactin secretion and successful therapy of the Forbes-Albright syndrome with L-Dopa. J. clin. Endocr. **34**, 306 (1972).
VARGA, L., WENNER, R., DEL POZO, E.: Treatment of galactorrhea amenorrhea syndrome with Br-ergocryptine (CB 154): restoration of ovulatory function and fertility. Amer. J. Obstet. Gynec. (in print).

Pituitary Gigantism

LOPIS, S., RUBINSTEIN, A.H., WRIGHT, A.D.: Measurements of serum growth hormone and insulin in gigantism. J. clin. Endocr. **28**, 393 (1968).
SOTOS, J.F., DODGE, P.R., MUIRHEAD, D., CRAWFORD, J.D., TALBOT, N.B.: Cerebral gigantism in childhood. New Engl. J. Med. **271**, 109 (1964).

Pathogenesis and Clinical Aspects of Acromegaly

ATKINSON, F.R.B.: Acromegaly. London: J. Bale & Danielsson 1932.
BAHAR, H., OPPIKOFER, A.M., LAMBERT, H.: Une forme particulière d'acromégalie: le syndrome de Troell-Junet. Helv. med. Acta, Suppl. **46**, 129 (1966).
BAILEY, P., CUSHING, H.: Studies in acromegaly; microscopical structure of adenomas in acromegalic dyspituitarism (fugitive acromegaly). Amer. J. Path. **4**, 545 (1928).
BECK, P., SCHALCH, D.S., PARKER, M.L., KIPNIS, D.M., DAUGHADAY, W.H.: Correlative studies of growth hormone and insulin plasma concentrations with metabolic abnormalities in acromegaly. J. Lab. clin. Med. **66**, 366 (1965).
BELL, H.N., BARTTER, F.C.: Studies of ^{47}Ca metabolism. J. clin. Endocr. **27**, 178 (1967).
BLICKENSTORFER, E.: Genealogie und Psychopathologie bei 51 Akromegalen. Acta endocr. (Kbh.) **13**, 123 (1953).
DAVIDOFF, L.M.: Studies in Acromegaly. III. The anamnesis and symptomatology in one hundred cases. Endocrinology **10**, 461 (1926).
— Hyperpituitarism and Hypopituitarism. Bull. N.Y. Acad. Med. **16**, 227 (1940).
ELIEL, L.P., PEARSON, O.H.: The metabolic effects of adrenocorticotropic hormone (ACTH) in a patient with Cushing's Syndrome and acromegaly. J. clin. Endocr. **11**, 913 (1951).
FRITZSCHE, E., KLEBS, E.: Ein Beitrag zur Pathologie des Riesenwuchses. Leipzig: F.C.W. Vogel 1884.
GORDON, D.A., HILL, F.M., EZRIN, C.: Acromegaly: a review of 100 cases. Canad med. Ass. J. **87**, 1106 (1962).
GORLIN, R.J., PINDBORG, J.J.: Idiopathic gingival fibromatosis and hypertrichosis. In: Syndromes of the head and neck. New York-Toronto-London: McGraw-Hill Book Comp. 1964.
HAMWI, G.J., SKILLMAN, TH.G., TUFTS, K.C., JR.: Acromegaly. Amer. J. Med. **29**, 690 (1960).
HOWARD, G.M., ENGLISH, F.P.: Occurrence of glaucoma in acromegalics. Arch. Ophth. **73**, 765 (1965).
IKKOS, D.: Pathophysiological studies in acromegaly. Pertaining to the extracellular water, renal function and basal metabolism. Acta endocr. (Kbh.) 21. Suppl. **25**, 1 (1956).
— LJUNGGREN, H., LUFT, R.: Glomerular filtration rate and renal plasma flow in acromegaly. Acta endocr. (Kbh.) **21**, 226 (1956).
— — — Basal metabolic rate in relation to body size and cell mass in acromegaly. Acta endocr. (Kbh.) **21**, 237 (1956).
JULIANI, G., MAURI, U.: Sul quadro radiologico delle alterazioni scheletriche nell'acromegalia. Radiol. med. (Torino) **48**, 542 (1962).
KELLER, H.H.: Zur Psychiatrie der Akromegalie. Dissertation Zürich 1949.
LANG, E.K., BESSLER, W.T.: The roentgenologic features of acromegaly. Amer. J. Roentgenol. **86**, 321 (1961).
LIM, N.Y., DINGMAN, J.F.: Androgenic adrenal hyperfunction in acromegaly. New Engl. J. Med. **271**, 1189 (1964).
LUFT, R., CERASI, E., HAMBERGER, C.A.: Studies on the pathogenesis of diabetes in acromegaly. Acta endocr. (Kbh.) **56**, 593 (1967).
MARGUTH, F., NOVER, A.: Morphologie und Klinik der Hypophysenadenom-Rezidive. Acta neurochir. (Wien) **11**, 716 (1963/64).
MAUTALEN, C.A., MELLINGER, R.C.: Nonsuppressible adrenocortical function in a patient with untreated acromegaly. J. clin. Endocr. **25**, 1423 (1965).
RIGOLOSI, R.S., SCHWARTZ, E., GLICK, S.M.: Growth hormone deficiency in acromegaly: A result of pituitary apoplexy. New Engl. J. Med. **279**, 362 (1968).
ROTH, J., GLICK, S.M., CUATRECASAS, P., HOLLANDER, CH.S.: Acromegaly and other disorders of growth hormone secretion. Ann. intern. Med. **66**, 760 (1967).
SÖNKSEN, P.H., GREENWOOD, F.C., ELLIS, J.P., LOWY, C., RUTHERFORD, A., NABARRO, J.D.N.: Changes of carbohydrate tolerance in acromegaly with progress of the disease and in response to treatment. J. clin. Endocr. **27**, 1418 (1967).

STEINER, H., DAHLBÄCK, K. O., WALDENSTRÖM, J.: Ectopic growth hormone production and osteoarthropathy in carcinoma of the bronchus. Lancet **1968 I**, 783.
STEWART, B. M.: The hypertrophic neuropathy of acromegaly. Arch. Neurol. (Chic.) **14**, 106 (1966).
SUMMERS, V. K., HUNTER, W. R., HIPKIN, L. J., DAVIS, J. C.: Hyperparathyroidism in acromegaly. Lancet **1966 II**, 601,
WRIGHT, A. D., HILL, D. M., LOWY, C., FRASER, T. R.: Mortality in acromegaly. Quart. J. Med., N.S. **39**, 1 (1970).
— MCLACHLAN, M. S., DOYLE, F. H., FRASER, T. R.: Serum growth hormone levels and size of pituitary tumor in untreated acromegaly. Brit. Med. J. **1969 II**, 346.

Treatment of Acromegaly

ALMQVIST, S., IKKOS, D., LUFT, R.: Studies on sulfation factor (SF) activity of human serum. The effects of oestrogen and x-ray therapy on serum SF activity in acromegaly. Acta endocr. (Kbh.) **37**, 138 (1961).
CROSS, J. N., GLYNNE, A., GROSSART, K. W. M., JENNETT, W. B., KELLETT, R. J., LAZARUS, J. H., THOMSON, J. A., WEBSTER, M. H. C.: Treatment of acromegaly by cryosurgery. Lancet **1972 I**, 215.
DASKE, A. M., SOLOMON, D. H., RAND, R. W., FRASIER, S. D., BROWN, J., SPEARS, I.: Stereotaxic hypophyseal cryosurgery in acromegaly and other disorders. J. Amer. med. Ass. **198**, 591 (1966).
DAUGHADAY, W. H. (Editorial): Acromegaly—a stubborn therapeutic challenge. New Engl. J. Med. **282**, 1430 (1970).
DRUCKER, W. D., SEGAL, B. M., VERDE, A. L., CHRISTY, N. P.: Adrenocorticotrophic activity in plasma of patients with acromegaly. Amer. J. Med. **43**, 383 (1967).
ELKINGTON, S. G., BUCKELL, M., JENKINS, J. S.: Endocrine function following treatment of pituitary adenoma. Acta endocr. (Kbh.) **55**, 146 (1967).
ESCHER, F.: Hypophysektomie. Fortschr. Hals-Nas.-Ohrenheilk. **12** (1965).
GREENWOOD, F. C., STEWART, H. J., FORREST, A. P. M., WOOD, R. G.: Plasma growth hormone levels in untreated acromegaly and after radioactive implants into the pituitary. Lancet **1965 II**, 555.
GUIOT, G.: Adénomes hypophysaires. Paris: Masson 1958.
HAMBERGER, C. A., HAMMER, G., NORLEN, G., SJÖGREN, B.: Surgical treatment of acromegaly. Acta oto-laryng. (Stockh.), (Suppl.) **158**, 168 (1960).
HARDY, J.: Transnasal-transphenoidal approach to the pituitary gland. In: YASARGIL, M. G.: Microsurgery applied to neurosurgery. Stuttgart: Thieme; New York-London: Academic Press 1969.
— ROBERT, F., SOMMA, M., VEZINA, J. L.: Acromégalie-gigantisme. Traitement chirurgical par exérèse transsphénoidale de l'adénome hypophysaire. Neurochirurgie 19, Suppl. **2** (1973).
HARTOG, M., DOYLE, F., FRASER, R., JOPLIN, G. F.: Partial pituitary ablation with implants of gold-198 and yttrium-90 for acromegaly. Brit. med. J. **1965 II**, 396.
HEIMBACH, S. B.: Follow-up studies on 105 cases of verified chromophobe and acidophile pituitary adenomata after treatment by transfrontal operation and x-ray irradiation. Acta neurochir. (Wien) **7**, 101 (1959).
HURXTHAL, L. M., HARE, H. F., HORRAX, G., POPPEN, J. L.: The treatment of acromegaly. J. clin. Endocr. **9**, 126 (1949).
KJELLBERG, R. N., SHINTANI, A., FRANTZ, A. G., KLIMAN, B.: Proton-beam therapy in acromegaly. New Engl. J. Med. **278**, 689 (1968).
KOLODNY, H. D., SHERMAN, L., SINGH, A., KIM, S., BENJAMIN, F.: Acromegaly treated with chlorpromazine. A case study. New Engl. J. Med. **284**, 819 (1971).
KOVACS, K., CARROL, R., TAPP, E.: Experimental hexadimethrine necrosis of anterior pituitary. Lancet **1964 II**, 919.
KOZAK, G. P., VAGNUCCI, A. I., LAULER, D. P., THORN, G. W.: Acromegaly pre- and postpituitary irradiation. Metabolism **15**, 290 (1966).
LAWRENCE, A. M., KIRSTEINS, L.: Progestines in the medical management of active acromegaly. J. clin. Endocr. **30**, 646 (1970).
— PINSKY, S. M., GOLDFINE, I. D.: Conventional radiation therapy in acromegaly. A Review and reassessment. Arch. intern. Med. **128**, 369 (1971).
LAWRENCE, J. H., TOBIAS, C. A., LINFOOT, J. A., BORN, J. L., LYMANM J. T., CHONG, C. Y., MANOUGIAN, E., WEI, W. C.: Successful treatment of acromegaly: metabolic and clinic studies in 145 patients. J. clin. Endocr. **31**, 180 (1970).
Leading article: Treatment of CUSHING's syndrome and acromegaly. Brit. med. J. **1965 II**, 379.
LINFOOT, J. A., GREENWOOD, F. C.: Growth hormone in acromegaly: effect of heavy particle pituitary irradiation. J. clin. Endocr. **25**, 1515 (1965).
MARGUTH, F., WILCKE, O.: Therapie der Hypophysenadenome. Dtsch. med. Wschr. **89**, 2029 (1964).
MCCULLAGH, E. P., BECK, J. C., SCHAFFENBURG, C. A.: Control of diabetes and other features of acromegaly following treatment with estrogens. Diabetes **4**, 13 (1955).
MOLINATTI, G. M., CAMANNI, F., MASSARA, F., OLIVETTI, M., PIZZINI, A., GIULIANI, G.: Implantation of $Yttrium^{90}$ in the sella turcica in sixteen cases of acromegaly. J. clin. Endocr. **22**, 599 (1962).
MUNDINGER, F., RIECHERT, T.: Hypophysentumoren, Hypophysektomie. Stuttgart: Thieme 1967.
NAGER, F. R.: Paranasal approach to intrasellar tumours. Semon lecture 1939. J. Laryng. **55**, 361 (1940).
RAND, R. W., DASKE, A. M., PAGLIA, D. E., CONVAY, L. W., SOLOMON, D. H.: Stereotatic cryohypophysectomy. J. Amer. med. Ass. **189**, 255 (1964).
RANDALL, R. V., CLARK, E. C., DODGE, H. W., JR., LOVE, J. G.: Polyuria after operation for tumours in the region of the hypophysis and hypothalamus. J. clin. Endocr. **20**, 1614 (1960).
RAY, B. S., HORWITH, M.: Surgical treatment of acromegaly. In: Clinical neurosurgery. Clin. Neurosurg. **10**, 31 (1964).
REIFENSTEIN, E. C., JR., KINSELL, L. W., ALBRIGHT, F.: Observations on the use of the serum phosphorus level as an index of pituitary growth hormone activity. The effect of estrogen therapy in acromegaly. J. clin. Endocr. **6**, 470 (1946).
ROTH, J., GORDEN, P., BATES, R. W.: Studies of growth hormone and prolactin in acromegaly. In: Growth hormone, ed. by PECILE and MÜLLER. Amsterdam: Excerpta med. Found. 1968.
— — BRACE, K.: Efficacy of conventional pituitary irradiation in acromegaly. New Engl. J. Med. **282**, 1385 (1970).
SHERMAN, L., KOLODNY, H. D.: The hypothalamus, braincatecholamines, and drug therapy for gigantism and acromegaly. Lancet **1971 I**, 682.
STUDER, H., LEHMANN, U.: Les problèmes endocrinologiques de l'hypophysectomie. Confin. neurol. (Basel) **23**, 275 (1963).
ZERVAS, N. T.: Technique of radio-frequency hypophysectomy. Confin. neurol. (Basel) **26**, 157 (1965).

Pathology and Clinical Aspects of Hormone-Inactive Pituitary Tumors

BAILEY, O. T., CUTLER, E. C.: Malignant adenomas of the chromophobe cells of the pituitary body. Arch. Path. **29**, 368 (1940).
BUCHBORN, E., IRMSCHER, K.: Diabetes insipidus bei Tumoren der Hypophyse. In: KRACHT, J., Oestrogene, Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

CAPLAN, R.H., DOBBEN, G.D.: Endocrine studies in patients with "empty sella syndrome". Arch. intern. Med. **123**, 611 (1969).

FEIRING, E.H., DAVIDOFF, L.M., ZIMMERMANN, H.M.: Primary carcinoma of the pituitary. J. Neuropath. exp. Neurol. **12**, 205 (1953).

FISCHER, P.A.: Hypophysenadenome, Psychopathologie und Endokrinologie. Stuttgart: F. Enke 1963.

FÜRST, E.: On chromophobe pituitary adenoma. A review of 131 cases. Acta med. scand., Suppl. **452** (1967).

GUBLER, R., STUDER, H.: Endokrine Störungen bei Hypophysentumoren. Helv. med. Acta **30**, 487 (1963).

JEFFERSON, M., ROSENTHAL, F.D.: Spontaneous necrosis in pituitary tumors. Lancet **1959 I**, 342.

JORES, A.: Nicht endokrin tätige Tumoren der Hypophyse und ihrer Nachbarschaft. In: Handbuch der inneren Medizin, 4. Aufl., Bd. 7, Teil I, S. 90. Berlin-Göttingen-Heidelberg: Springer 1955.

KAPPELER, R.: Hyperthyreose und Panhypopituitarismus im Verlauf eines chromophoben Adenoms der Hypophyse. Schweiz. med. Wschr. **89**, 367 (1959).

KERNOHAN, J.W., SAYRE, G.P.: Tumors of the pituitary gland and infundibulum. Atlas of tumor pathology, sect. X, fasic. 36. Washington, D.C.: Armed Forces Inst. Pathology 1956.

KRACHT, J., HACHMEISTER, U.: Hormonbildungsstätten im Hypophysenvorderlappen des Menschen. In: KRACHT, J., Oestrogene, Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

Leading article: The "empty" sella. Brit. med. J. **1970 II**, 679.

LOCKE, S., TYLER, R.: Pituitary apoplexy. Amer. J. Med. **30**, 643 (1961).

MARGUTH, F., FAHLBUSCH, R.: Chirurgie der Hypophysentumoren. In: KRACHT, J., Oestrogene, Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

MARKS, V., SUMMERS, M.: Pituitary-adrenal function in cases of pituitary tumor. Brit. med. J. **1963 II**, 155.

MOGENSEN, E.F.: Chromophobe adenoma of the pituitary gland. Acta endocr. (Kbh.) **24**, 135 (1957).

MÜLLER, W.: Pathologie der Hypophysentumoren. In: KRACHT, J., Oestrogene Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

NIEMAN, E.A., LANDON, J., WYNN, V.: Endocrine function in patients with untreated chromophobe adenomas. Quart. J. Med. **36**, 357 (1967).

OBERDISSE, K., TÖNNIS, W.: Pathogenese, Klinik und Behandlung der Hypophysenadenome. Ergebn. inn. Med. Kinderheilk. **4**, 975 (1953).

SCHWARZ, K.: Pathophysiologie und Klinik der Hypophysentumoren. In: KRACHT, J., Oestrogene, Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

SOLBACH, H.G., BETHGE, H., ZIMMERMANN, H.: Funktionsdiagnostik der Hypophysentumoren. In: KRACHT, J., Oestrogene, Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

WHITE, J.C., BALLANTINE, H.T., JR.: Intrasellar aneurysms simulating hypophyseal tumors. J. Neurosurg. **18**, 34 (1961).

WRIGHT, R.L., OJEMANN, R.G., DREW, J.H.: Hemorrhage into pituitary adenomata. Arch. Neurol. (Chic.) **12**, 326 (1965).

YOUNGHOUSBAND, O.Z., HORRAX, G., HURXTHAL, L.M., HARE, H.F., POPPEN, J.L.: Chromophobe pituitary tumors. I. Diagnosis. J. clin. Endocr. **12**, 611 (1952).

ZÜLCH, K.J.: Biologie und Pathologie der Hirngeschwülste. Handbuch der Neurochirurgie. Hrsg. von H. OLIVECRONA u. W. TÖNNIS, Bd. III, S. 1. Berlin-Göttingen-Heidelberg: Springer 1956.

Treatment of Endocrine-Inactive Pituitary Tumors

BAKAY, L.: The results of 300 pituitary adenoma operations. J. Neurosurg. **7**, 240 (1950).

DECOURT, J., PETIT-DUTAILLIS, D., MICHARD, J.P., BERNARD-WEIL, E.: Progres apportés aux résultats du traitement chirurgical des adénomes de l'hypophyse et des craniopharyngiomes par l'hormonothérapie cortico-surrénalienne pré- et postopératoire. Presse méd. **43**, 971 (1958).

ELKINGTON, S.G., BUCKELL, M., JENKINS, J.S.: Endocrine function following treatment of pituitary adenoma. Acta endocr. (Kbh.) **55**, 146 (1967).

— MCKISSOCK, W.: Pituitary adenoma: results of combined surgical and radiotherapeutic treatment of 260 patients. Brit. med. J. **1967 I**, 263.

GURDJIAN, E.S., WEBSTER, J.E., LATIMER, F.R., KLEIN, S.P., LOFSTROM, J.E.: Recent advances in surgical management of chromophobe tumor of pituitary. J. Amer. med. Ass. **158**, 23 (1955).

HORRAX, G., HARE, H.F., POPPEN, J.L., HURXTHAL, L.M., YOUNGHOUSBAND, O.Z.: Chromophobe pituitary tumors. II. Treatment. J. clin. Endocr. **12**, 631 (1952).

— SMEDAL, M.I., TRUMP, J.G., GRANKE, R.C., WRIGHT, K.A.: Present day treatment of pituitary adenomas. Surgery versus x-ray therapy. New Engl. J. Med. **252**, 524 (1955).

MATSON, D.D.: Craniopharyngioma. Clinical neurosurgery, vol. 10. Baltimore: Williams & Wilkins 1964.

MUNDINGER, F., RIECHERT, T.: Hypophysentumoren, Hypophysektomie. Stuttgart: Thieme 1967.

OBERDISSE, K., TÖNNIS, W.: Pathogenese, Klinik und Behandlung der Hypophysenadenome. Ergebn. inn. Med. Kinderheilk. **4**, 975 (1953).

SCRIBA, P.C.: Postoperative Diagnostik und Substitutionstherapie bei Hypophysentumoren. In: KRACHT, J., Oestrogene, Hypophysentumoren. Berlin-Heidelberg-New York: Springer 1969.

TÖNNIS, W., OBERDISSE, K., WEBER, E.: Bericht über 164 operierte Hypophysenadenome. Acta neurochir. (Wien) **3**, 113 (1953).

TROEN, P., RYNEARSON, E.H.: The prophylactic use of cortisone for surgery of the pituitary. J. clin. Endocr. **15**, 857 (1955).

Diagnosis of Adenohypophyseal Function

BECK, P., SCHALCH, D.S., PARKER, M.L., KIPNIS, D.M., DAUGHADAY, W.H.: Correlative studies of growth hormone and insulin plasma concentrations with metabolic abnormalities in acromegaly. J. Lab. clin. Med. **66**, 366 (1965).

BENOIT, F.I., THEIL, G.B., WATTEN, R.H.: Hydroxyproline excretion in endocrine disease. Metabolism **12**, 1072 (1963).

BODEN, G., SOELDNER, J.S.: A sensitive double antibody radioimmunoassay for human growth hormone (HGH): Levels of serum HGH following rapid tolbutamid infusion. Diabetologia **3**, 413 (1967).

— — STEINKE, J., THORN, G.W.: Serum human growth hormone (HGH) response to i.v. glucose: Diagnosis of acromegaly in females and males. Metabolism **17**, 1 (1968).

CERASI, E., DELLA CASA, L., LUFT, R., ROOVETE, A.: Determination of human growth hormone (HGH) in plasma by a double antibody radioimmunoassay. Acta endocr. (Kbh.) **53**, 101 (1966).

CRYER, PH.E., E., DAUGHADAY, W.H.: Regulation of growth hormone secretion in acromegaly. J. clin. Endocr. **29**, 386 (1969).

DAUGHADAY, W.H.: The diagnosis of hypersomatotropism in man. Med. clin. N. Amer. **52**, 371 (1968).

DAUGHADAY, W. H.: Growth hormone assay in acromegaly, gigantism, dwarfism and hypopituitarism. Postgrad. Med. **46**, 84 (1969).

— SALMON, W. D., JR., ALEXANDER, F.: Sulfation factor activity of sera from patients with pituitary disorders. J. clin. Endocr. **19**, 743 (1959).

DULL, T. A., HENNEMAN, PH. H.: Urinary hydroxyproline as an index of collagen turnover in bone. New Engl. J. Med. **268**, 132 (1963).

EARLL, J. M., SPARKS, L. L., FORSHAM, P. H.: Glucose suppression of serum growth hormone in the diagnosis of acromegaly. J. Amer. med. Ass. **201**, 628 (1967).

EFRON, M. L., BIXBY, E. M., PRYLES, CH. V.: Hydroxyprolinemia. II. A rare metabolic disease due to a deficiency of the enzyme "Hydroxyproline Oxidase". New Engl. J. Med. **272**, 1299 (1965).

ERDHEIM, J.: Die akromegalen Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie. Berlin-Wien: Springer 1931.

FRANTZ, A. G., RABKIN, M. T.: Human growth hormone. Clinical measurement, response to hypoglycemia and suppression by corticosteroids. New Engl. J. Med. **271**, 1375 (1964).

FRASER, R., WRIGHT, A. D.: Standard procedures for assessing hypersecretion or secretory capacity for human growth hormone using the radioimmuno assay. Postgrad. med. J. **44**, 53 (1968).

GEMZELL, C. A.: Demonstration of growth hormone in human plasma. J. clin. Endocr. **19**, 1049 (1959).

GLICK, S. M., ROTH, J., YALOW, R. S., BERSON, S. A.: Immunoassay of growth hormone in plasma. Nature (Lond.) **199**, 784 (1963).

GREENWOOD, F. C.: Growth hormone. In: GRAY, C. H., and A. L. BACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

HUNTER, W. M., GREENWOOD, F. C.: A radio-immunoelectrophoretic assay for human growth hormone. Biochem. J. **91**, 43 (1964).

JASIN, H. E., FINK, C. W., WISE, W., ZIFF, M.: Relationship between urinary hydroxyproline and growth. J. clin. Invest. **41**, 1928 (1962).

KAPLAN, S. L., ABRAMS, C. A. L., BILL, J. J., CONTE, F. A., GRUMBACH, M. M.: Growth and growth hormone. I. Changes in serum level of growth hormone following hypoglycemia in 134 children with growth retardation. Pediat. Res. **2**, 43 (1968).

MERIMEE, T. J., RABINOWITZ, D., RIGGS, L., BURGESS, J. A., RIMOIN, D. L., MCKUSICK, V. A.: Plasma growth hormone after arginine infusion. New Engl. J. Med. **276**, 434 (1967).

MITCHELL, M. L., BYRNE, M. J., SANCHEZ, Y., SAWIN, C. T.: Detection of growth hormone deficiency. The glucagon stimulation test. New Engl. J. Med. **282**, 539 (1970).

PARKER, M. L., HAMMOND, J. M., DAUGHADAY, W. H.: The arginine provocative test: and aid in the diagnosis of hyposomatotropism. J. clin. Endocr. **27**, 1129 (1967).

PENNY, R., BLIZZARD, R. M., DAVIS, W. T: Sequential study of arginine monochloride and normal saline as stimuli to growth hormone release. Metabolism **19**, 165 (1970).

PROCKOP, D. J., KIVIRIKKO, K. I.: Relationship of hydroxyproline excretion to collagen metabolism. Ann. intern. Med. **66**, 1243 (1967).

— UDENFRIEND, S.: Specific method for analysis of hydroxyproline in tissues and urine. Analyt. Biochem. **1**, 228 (1960).

ROTH, J., GLICK, S. M., CUATRECASAS, P., HOLLANDER, CH. S.: Acromegaly and other disorders of growth hormone secretion. Ann. intern. Med. **66**, 760 (1967).

SALINAS, A., MÖNCKEBERG, F., BEAS, F.: Immunological detection of growth hormone in normal human urine. Lancet **1963 II**, 302.

SMILEY, J. D., ZIFF, M.: Urinary hydroxyproline excretion and growth. Physiol. Rev. **44**, 30 (1964).

SULLIVAN, C. R., JONES, D. R., BAHN, R. C., RANDALL, R. V.: Biopsy of the costochondral junction in acromegaly. Proc. Mayo Clin. **38**, 81 (1963).

UTIGER, R. D.: Extraction and radioimmunoassay of growth hormone in human serum. J. clin. Endocr. **24**, 60 (1964).

— PARKER, M. L., DAUGHADAY, W. H.: Studies on human growth hormone. I. A radioimmunoassay for human growth hormone. J. clin. Invest. **41**, 254 (1962).

Added in Proofs:

HORROBIN, D. F.: Prolactin: Physiology and clinical significance. Lancaster: MTP Medical and Technical Publishing Co. Ltd. (1973).

HALL, R., BESSER, G. M., SCHALLY, A. V., COY, D. H., EVERED, D., GOLDIE, D. J., KASTIN, A. J., MCNEILLY, A. S., MORTIMER, C. H., PHENEKOS, C., TUNBRIDGE, W. M. G., WEIGHTMAN, D.: Action of growth-hormone-release inhibitory hormone in healthy men and in acromegaly. Lancet **1973 II**, 581.

LAMBERG, B.-A., RIPATTI, J., GORDIN, A., JUUSTILA, H., SIVULA, A., BJÖRKESTEN, G. af: Chromophobe pituitary adenoma with acromegaly and TSH-induced hyperthyroidism associated with parathyroid adenoma. Acta endocr. (Kph.) **60**, 157 (1969) (see also Chap. VI, p. 209).

VI. The Thyroid Gland

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With Contributions by

CHR. HEDINGER, G. KISTLER, A. PRADER, and G. TÖNDURY

A. Historical Dates

1543 VESAL described the thyroid gland.
1606 PARACELSUS related cretinism to the thyroid gland (published posthumously).
1786 PARRY described hyperthyroidism (published posthumously 1825).
1802 FLAIANI described hyperthyroidism.
1820 COINDET introduced iodine into the treatment of goiter.
1833 BOUSSINGAULT recommended iodine for the prevention of goiter.
1835 GRAVES described hyperthyroidism.
1836 KING assumed the presence of an internal secretion of the thyroid.
1840 BASEDOW gave a classic description of the Merseburg triad (exophthalmos, goiter and tachycardia).
1852 CHATIN justified the theory that iodine deficiency caused goiter, by proving a diminished iodine content of the drinking water in endemic areas.
1873 GULL described the classic picture of myxedema.
1878 ORD related it to atrophy of the thyroid gland.
1882 First description of the complications of thyroidectomy in man by the REVERDIN brothers.
1883 KOCHER described "cachexia strumipriva" (postoperative myxedema).
1886 MOEBIUS found that Graves' disease was due to hyperfunction of the thyroid gland.
1888 Report of the Myxedema Commission of the Clinical Society of London.
1891 MURRAY successfully treated myxedema with injections of thyroid extract.
1893 FRIEDRICH VON MÜLLER demonstrated a negative metabolic balance in hyperthyroidism.
1895 MAGNUS-LEVY recognized that the oxygen consumption at rest was elevated in thyrotoxicosis and reduced in myxedema and that the basal metabolic rate following the administration of desiccated tissue was increased.
1896 BAUMANN showed that iodine was a constituent of the thyroid gland.
1899 OSWALD isolated thyroglobulin as a component of the thyroid.
1910 MARINE and LENHART succeeded in preventing goiter by using iodine in animal experiments.
1912 GUDERNATSCH recognized the acceleration of metamorphosis caused by thyroxine.
1913 PLUMMER described the toxic adenoma.
1914 KENDALL succeeded in purifying and crystallizing the thyroid hormone, thyroxine.
1917 First successful iodine-prophylaxis of goiter in man by MARINE and KIMBALL.
1918 AUB, DUBOIS, BENEDICT, and HARRIS introduced the measurement of basal metabolic rate as a clinical method.
1922 PLUMMER introduced iodine treatment for hyperthyroidism.
1926 HARINGTON established the chemical constitution of thyroxine.
1927 HARINGTON and BARGER synthesized thyroxine.
1927 SMITH showed the existence of thyrotropin in the pituitary gland.
1928 CHESNEY, CLAWSON and WEBSTER discovered goitrogenic substances in different types of cabbage.
1940 Introduction of radioactive iodine for the clinical evaluation of thyroid function by HERTZ, ROBERTS, MEANS, and EVANS.
1942 ASTWOOD systematically examined goitrogenic substances and introduced thiouracil into the treatment of hyperthyroidism.
1942 HAMILTON and LAWRENCE introduced radioactive iodine into the treatment of the thyroid gland.
1952 Discovery of triiodothyronine by GROSS and PITT-RIVERS and ROCHE, MICHEL, and LISSITZKY.
1956 ADAMS, PURVES, and MCKENZIE independently found the long-acting thyroid stimulator (LATS) in the serum of thyrotoxic patients.

1956 ROITT, DONIACH, CAMPBELL, and HUDSON discovered thyroid antibodies in Hashimoto thyroiditis.
1970 GUILLEMIN and SCHALLY successfully isolated thyrotropin releasing hormone (TRH) from hypothalamic extracts.
1970 GILLESSEN and co-workers established the chemical constitution of TRH and synthesized the hormone.

B. Embryology, Gross Anatomy, and Histology

G. TÖNDURY and G. KISTLER

Embryology. The anlage of the thyroid gland, like that of the hypophyseal glandular lobe, appears very early in embryonic life. In the human embryo 3.5 to 4 mm in length, an entodermal outpocketing forms in the floor of the oral cavity, just beneath the buccopharyngeal membrane and between the first pair of pharyngeal pouches. At the bottom of this small pit an epithelial bud protrudes into the underlying mesenchyma. The primordium becomes displaced caudally, but remains attached to the floor of the pharynx by the *thyroglossal duct*. During this descensus, the originally compact bud becomes bilobed and differentiates into a mass of irregularly arranged epithelial cords, which later form the isthmus and the lateral lobes of the gland. When the primordium reaches the level of the uppermost tracheal cartilage, the thyroglossal duct becomes fragmented. A *cranial* fragment, the *lingual duct*, remains attached to the tongue initially but usually disappears at later stages. Occasionally however, salivary gland-like structures and small colloid-filled follicles develop from its epithelium. The point of origin of the duct forms the *foramen cecum*, an enlarged pit which persists, in about 50% of the population, in the midpoint of the sulcus terminalis between dorsum and radix linguae. The *distal* fragment of the thyroglossal duct gives rise to the pyramidal lobe. In the eighth week, small rounded or elongated cavities begin to appear in the thyroid cords. They develop into the primary thyroid follicles, which acquire colloid at the beginning of the third gestational month and become functional soon afterwards (GREENBERG, 1970).

Gross Anatomy. The thyroid gland consists of a left and a right lobe which are connected across the median plane by the narrow isthmus. The lateral lobes are conical in shape. The base is usually on a level with the fourth or fifth tracheal ring. The apex, which is directed upwards, usually reaches the oblique line of the thyroid cartilage. The isthmus usually covers the second and third tracheal rings, but may also be higher or lower. The pyramidal lobe varies widely in size and shape, occasionally extending up to the hyoid bone. The gland is situated at the level of the fifth to seventh cervical and the first thoracic vertebrae. Its medial surface is in close contact with the larynx and trachea. Its posterolateral surfaces are related to the esophagus and the neurovascular tracts of the neck. Anteriorly, the thyroid is separated from the sternothyroid muscles by the pretracheal fascia. A connective tissue capsule is closely applied to and inseparable from the glandular parenchyma. An additional, outer and thicker capsule encloses the larger branches of the thyroid blood vessels and the parathyroid glands. Small aberrant fragments of thyroid tissue are sometimes found outside this outer capsule, mostly along the obliterated thyroglossal duct (so-called accessory thyroid glands).

The thyroid gland is a highly vascular organ. It is usually supplied by two pairs of arteries, the branches of which anastomose freely with each other. The *superior* thyroid artery arises on each side as the first branch of the external carotid. It supplies the upper, anterior, and lateral portions of the lateral lobes. The *inferior* thyroid artery is a branch of the thyrocervical trunk which in turn arises from the first portion of the subclavian artery. It supplies the inferior, posterior and medial parts of the organ on either side. In its course to the thyroid gland, the inferior thyroid artery crosses the *recurrent laryngeal nerve* of the vagus and the *sympathetic trunk*, which often forms a loop around it, the ansa thyreoidea. The recurrent laryngeal nerve ascends, on the left side, in a groove between the trachea and the esophagus, and, on the right side, along the trachea. At the level of the first tracheal ring, it gives off the inferior laryngeal nerve which runs in front of, or behind the branches of the inferior thyroid artery to supply the intrinsic muscles of the larynx. Ligation of the arteries during thyroidectomy therefore involves some hazard to the recurrent laryngeal nerve. Occasionally, there is a fifth artery, the unpaired *arteria thyreoidea ima*, which arises from the arch of the aorta or from the brachiocephalic trunk and ascends to the isthmus. The veins form a rich network on the surface of the organ and on the front of the trachea. From this plexus, a superior, a middle and an inferior thyroid vein arise. The former two end in the internal jugular vein, the latter in the innominate vein. A dense capillary network surrounds the thyroid follicles. Closely applied to the capillaries are numerous branching lymph vessels which run

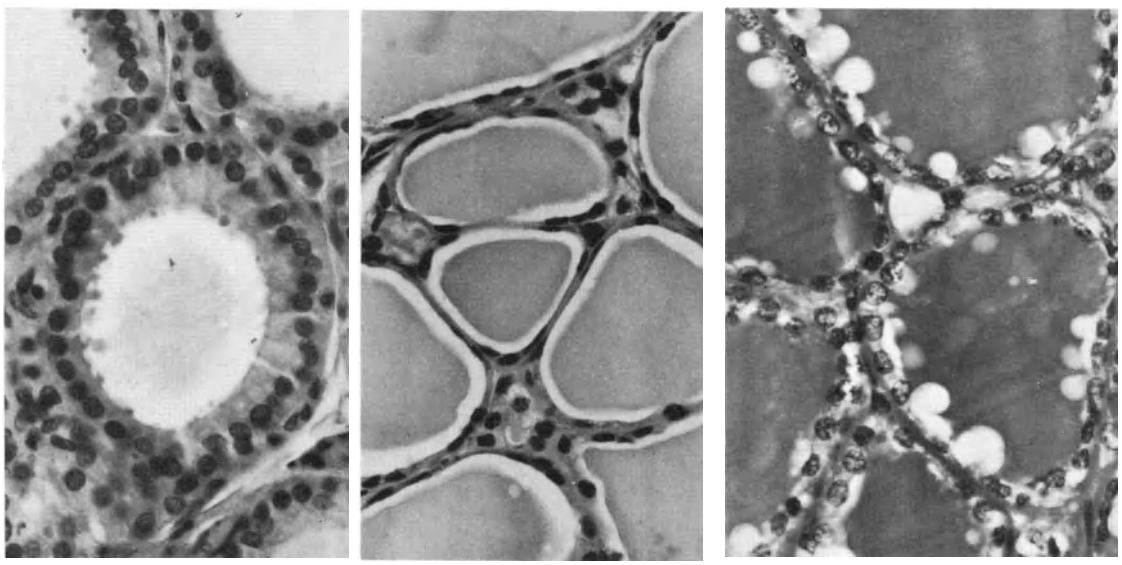
in the interfollicular connective tissue, often along the arterioles, to a network in the capsule of the gland. From here, larger lymph vessels extend to the thoracic duct and to the right lymphatic duct.

Numerous autonomous nerve fibers derived from the middle and inferior cervical ganglia of the sympathetic trunk penetrate the capsule, mostly together with the arteries. In the interfollicular spaces, they form a dense network of nonmyelinated nerve fibers, whose endings terminate in close proximity to the follicles.

Histology. At an embryonic age of about eight weeks, the epithelial cords of the thyroid primordium disintegrate into small vesicles, the *primary* or two-cell follicles, which contain small amounts of colloidal material. From these, rounded or rod-shaped *secondary* (definitive) follicles sprout out and enlarge. The follicles of the mature gland measure up to 1 mm in diameter. They contain a gelatinous colloid and are lined by a simple epithelium, the depth of which varies widely according to the functional state of the gland. At the peak of *secretory* activity (Fig. 1a), the epithelial cells are columnar in shape. Mitochondria and numerous clear vesicles appear to accumulate in the apical portions of the cells and the Golgi complex is found in a supranuclear position. The colloid produced in the cells is secreted into the follicle cavity, where it gradually becomes concentrated. At this point, the epithelial cells assume a cuboid or flattened shape (Fig. 1b) corresponding to a resting state. *Resorption* of the colloid from its storage site seems to be initiated by liquefaction of its outermost portions. The cells again become cuboidal or columnar and their cytoplasm turns foamy (Fig. 1c). Empty follicles are characterized by their star-shaped lumen. It is assumed that they are able to refill the follicle cavity with colloid within a few hours.

Each follicle is enclosed by a very thin basal membrane and a delicate network of reticular fibers. In addition, it is surrounded by a capillary plexus and by numerous blind terminations of lymphatic vessels. The interstitial arterial network of the thyroid gland is rich in regulatory structures such as subendothelial bundles of epitheloid muscle cells, as well as arteriovenous anastomoses. Veins and larger lymphatics accompany the arteries. A large number of sympathetic nerve fibers originate in the middle and superior cervical ganglia and run along the larger blood vessels; they are presumed to be vasomotor.

A population of larger cells which do not stain as well is regularly observed in both the follicular epithelium and the interfollicular spaces. When stained by the silver nitrate method of CAJAL, these so-called *parafollicular cells* (mitochondria-rich cells, C cells) display a large number of small brown or black granules. As recent investigations have shown, these cells produce the hormone thyrocalcitonin (Chap. XIV).



a b c

Fig. 1a–c. Morphologic variations of the epithelial cells depending on the functional state of the thyroid gland. a) secretory phase, columnar cells; b) resting state, flattened epithelium; c) resorption of the colloid, cuboidal to columnar epithelial cells and vacuolization of the colloid within the follicle cavity. [With permission of Prof. CHR. HEDINGER: Normale und pathologische Anatomie der Schilddrüse, Verh. dtsch. Ges. inn. Med. **66,** 13 (1960)]

Electron microscopy reveals a large number of *microvilli* and occasional single cilia at the apical surface of the epithelial cells. Their shape varies widely according to the functional state of the follicle. The microvilli of resting cells are short and straight; those of secreting or resorbing cells appear larger and pleomorphic. Laterally, the follicle cells are joined by typical junctional complexes. Some small infoldings of the plasma membrane are regularly observed in the basal regions of the epithelium. In the active cell, the Golgi apparatus is composed of flattened saccules and numerous vesicles and vacuoles. It is usually situated above the relatively large nucleus which occupies the basal cytoplasmic region. The endoplasmic reticulum consists of elongated, rough-surfaced cisternae and a large number of small vesicles. Ovoid or rounded mitochondria, multivesicular bodies, and vacuoles of varying size are randomly distributed within the cytoplasmic ground substance.

The uptake of iodine from the blood plasma and its oxidation within the epithelial cells are discussed on p. 139. Independently of these processes, which are still poorly understood, thyroglobulin fragments are synthesized on the ribosomes of the granular endoplasmic reticulum. They are carried within small vesicles to the Golgi region, where they seem to be coupled to carbohydrates (NADLER, 1964). The vesicles then reach the apical surface of the epithelial cell and their membrane fuses with the plasma membrane. Iodination of the thyroglobulin seems to occur at the moment when the content of the vesicle is emptied into the follicle cavity (Fig. 3). Resorption of the thyroglobulin-bound hormone molecules takes place again at the apical surface of the cells, where colloid droplets of varying size are found to be phagocytozed. While, in resting cells, the lysosomes are randomly distributed throughout the cytoplasm, in cells engaged in resorption they accumulate in the apical portions of the cytoplasm, where they are observed to fuse with the phagocytozed colloid droplets. After breakdown of the thyroglobulin by the hydrolytic enzymes, the hormones thyroxine and triiodothyronine are carried in molecular form to the basal portions of the epithelial cells and reach the capillary network and the terminations of the lymphatic vessels by a yet unknown pathway.

C. Biochemistry and Physiology

1. Chemistry of the Thyroid Hormones

The thyroid gland secretes two iodinated amino acids with hormonal activity, L-thyroxine (T_4) and 3,5,3′-L-triiodothyronine (T_3). The structural formulas are given in Fig. 2. The two benzene rings are joined by an ether linkage which forms an angle of 110° at the oxygen atom. The three-dimensional structure of thyroxine and triiodothyronine have recently been established by X-ray crystallographic analysis (CAMERMAN, 1972). The two benzene rings are in planes almost perpendicular to each other and the four iodine atoms of thyroxine form the apices of a distorted tetrahedron.

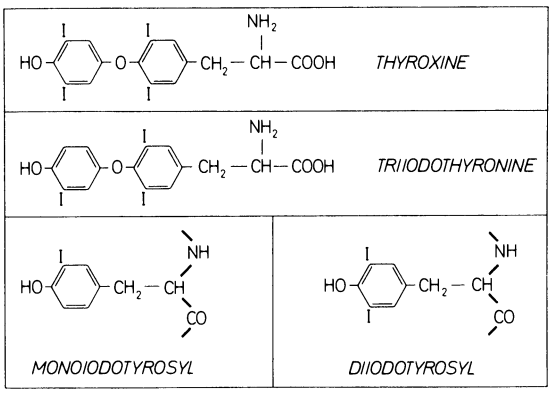


Fig. 2. Chemical formulas of thyroid hormones and their precursors. Mono- and diiodotyrosine are shown as amino acid residues, since they occur mainly as part of the protein thyroglobulin. The peptide linkages are shown by thick lines

Thyroxine and triiodothyronine are poorly soluble in water at neutral pH. They are soluble in 0.1 N NaOH and also in ethanol or methanol rendered alkaline by ammonia.

The natural compounds are levorotatory. The dextrorotatory stereoisomers have only a weak thyromimetic activity of about 1/20 that of the natural hormones. It has been said that D-thyroxine differs qualitatively in its effects from L-thyroxine, and in particular that it lowers serum lipids much more than it affects oxygen consumption and basal metabolic rate (BANSI, 1962). In view of the fact that D-thyroxine had to be given in high doses to lower serum lipids and that the preparations tested may have contained appreciable quantities of L-thyroxine as a contaminant, these claims are certainly open to question.

Numerous hormone analogues have been prepared, and some have been found to be hormonally active, although in most cases less so than the parent compound when compared on a weight basis (MONEY, 1960; JØRGENSEN, 1971) but some, such as 3'-isopropyl-3,5-diiodothyronine are definitely more active than triiodothyronine. Natural breakdown products of tyroid hormones, such as tetraiodothyroacetic acid

(tetrac) and triiodopropionic acid (triprop), are also hormonally active. Their exact physiological activity is difficult to evaluate as they have different half-lives, membrane permeabilities and distribution volumes. The requirements for thyroid hormone activity of an analogue have been specified as follows: 1. the thyronine skeleton; 2. diiodo- or dibromo substitutions in the 3 and 5 positions; 3. bulky substitutions (e.g. iodine, isopropyl) in the 3′ position (BARKER, 1971).

2. Hormone Biosynthesis and Release

Dietary iodine intake shows wide geographic and day-to-day variations. In regions of iodine deficiency it can be as low as 10 µg per day. In the U.S.A. the daily intake varies between about 100 and 1000 µg (ODDIE, 1970) and there are reliable data to show that iodine consumption is rising (PITTMAN, 1969). 100 µg per day is considered a sufficient, 200 µg an optimal iodine supply. Most of the dietary iodine is supplied as iodide, and some as organic or molecular iodine. The latter is reduced to iodide during intestinal absorption. The plasma level of inorganic iodide depends largely on the dietary intake and is between 0.1 and about 1.0 µg/100 ml. The extrathyroidal iodide space in man is about 38% of body weight. Iodide is filtered in the renal glomerula and partially reabsorbed in the renal tubuli. The renal clearance is 15 to 55 ml/min, with an average of 35 ml. It is diminished in uremia, but otherwise the renal iodide clearance is quite constant in a given subject. Gastric mucosa and the salivary glands are extrathyroidal tissues which can concentrate iodide. The iodide concentrated in this way is secreted back into the alimentary tract and is reabsorbed in the gut. Substantial amounts of iodide may be lost in the breast milk.

a) Thyroidal Iodide Uptake

Besides the kidney, the only organ clearing significant amounts of iodide from the blood is the thyroid (Fig. 4). Unlike the renal iodide clearance, which is very constant, the thyroidal clearance is highly adaptable. On average it amounts to about 25 ml/min, but in conditions of iodine deficiency or of thyroid gland hyperfunction the clearance rises to over 100 ml/min. Iodide is actively transported across the basal thyroid cell membrane against an electrochemical concentration gradient (step 1, Fig. 3). The concentration within the cell is 5 to 300 times higher than that in the plasma, the exact value depending on the functional state of the gland. Iodide transport requires energy, which is supplied by ATP. Inhibition of membrane-bound ATPase by ouabain abolishes active

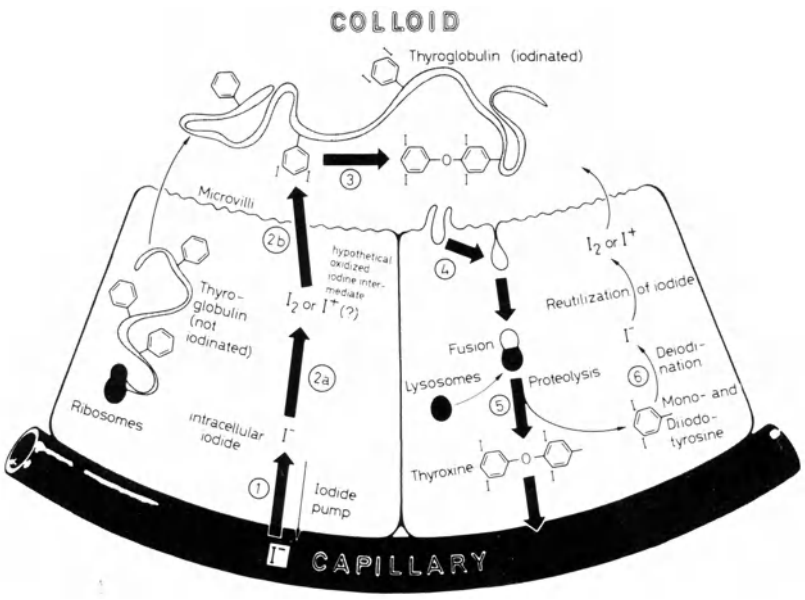


Fig. 3. Schematic representation of thyroid hormone biosynthesis and release. The individual steps are numbered as follows: 1. Active transport of iodide from the blood into the thyroid cell. 2a. Oxidation of the intrathyroidal iodide by a peroxidase to an as yet unidentified oxidized iodine intermediate. 2b. Transfer of this oxidized iodine intermediate to a tyrosyl residue of thyroglobulin. 3. Coupling of two diiodotyrosyl residues within thyroglobulin to form thyroxine. 4. Endocytosis of colloid. 5. Fusion of colloid droplets with lysosomes. Hydrolysis of thyroglobulin with release of free thyroxine and free iodotyrosines. 6. The free iodotyrosines are deiodinated within the gland by a specific deiodinase. The iodide thus recovered is reincorporated into thyroglobulin

iodide transport (WOLFF, 1964). Several authors have postulated the existence of a specific iodide carrier, but conclusive isolation has not been achieved (see WOLFF, 1964, for review). TSH enhances active iodide transport after a lag of several hours. On the other hand, active iodide transport may also be controlled by extrapituitary factors, probably involving so-called thyroid autoregulation. Several monovalent anions specifically inhibit iodide transport. The most potent of these is perchlorate (ClO_4^-). Others include nitrate (NO_3^-) and thiocyanate (CNS^-). They are thought to act by competitive inhibition of the active iodide transport, but in the case of thiocyanate the mechanism may be more complex and is not completely clear. Iodide transported into the cell is rapidly metabolized to organic iodine. In normal circumstances the process of organic iodination is so efficient that inorganic iodide concentration in the gland remains very low. Therefore the rate-limiting step in the synthesis of organic iodine is that of transport of iodide. When organic iodination fails for some reason, iodide can leave the gland again, probably by simple passive diffusion. A second source of thyroidal iodide is mono- and diiodotyrosine, two amino acids resulting from thyroglobulin hydrolysis. They are quantitatively deiodinated within the gland by a potent deiodinase, and most of the iodide produced is reutilized for organic iodination. There is some evidence that this so-called endogenous iodide is confined to a different compartment than the iodide transported from outside (second iodide pool; HALMI, 1962; NAGATAKI, 1963).

b) Organic Iodination

The iodide transported into the cell is rapidly incorporated into thyroglobulin (step 2a and 2b, Fig. 3). It must be stressed here that the iodination takes place on the tyrosyl residues of the protein, and not on the free amino acid tyrosine. The sites of substitution are the carbon atoms number 3 or 5 of the tyrosyl ring. When one atom of iodine is substituted, monoiodotyrosyl is formed, with two iodine atoms diiodotyrosyl. The cellular site of the reaction is still a matter of dispute. By radioautography, injected radioiodine can be detected in organic form in the lumen of the follicles within seconds after the injection (NADLER, 1955), which was earlier used as an argument that iodination of thyroglobulin occurred in the follicular lumen. More recently it has been demonstrated that certain cellular organelles are capable of carrying out organic iodination, and the process is now located by most authorities at the interphase between cell and colloid. The cell membrane contains numerous microvilli, which might be the organelles involved in iodination (BEN-ABDELJLIL, 1967). Iodide as such cannot be incorporated into thyroglobulin. Rather it must

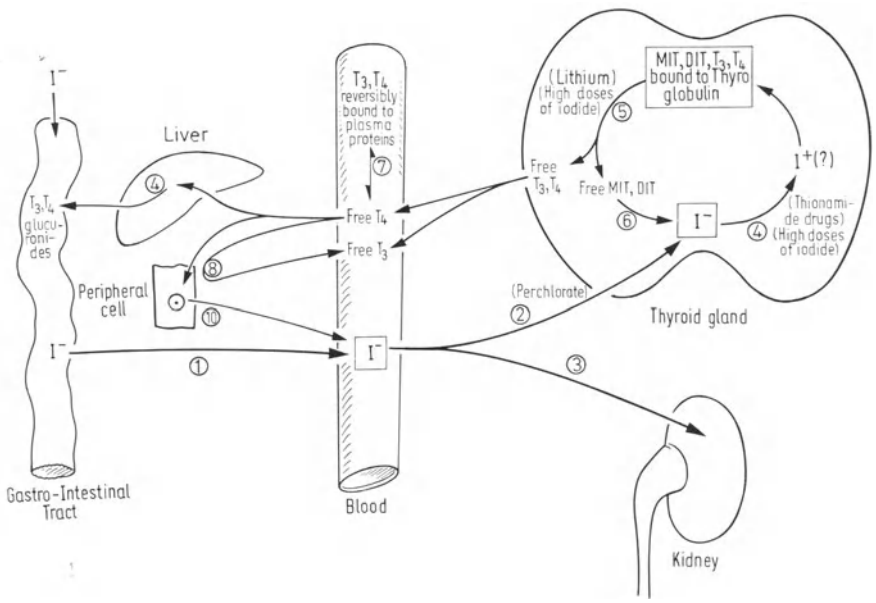


Fig. 4. Main pathways of iodine and thyroid hormone metabolism. 1. Uptake of iodide from the gastrointestinal tract. 2 and 3. Clearance of iodide from the blood by the thyroid and the kidney. 4. Incorporation of iodide into organic compounds of thyroglobulin. 5. Hydrolysis of thyroglobulin. 6. Deiodination of iodotyrosines. 7. Reversible protein binding of thyroid hormones to specific proteins of plasma. 8. Peripheral conversion of thyroxine into triiodothyronine. 9. Conjugation of thyroid hormones with glucuronide in the liver. 10. Deiodination of thyroid hormones by peripheral cells. Inhibitory drugs are indicated in brackets

be oxidized to some more electrophilic intermediate substance. Despite intensive research the exact nature of the iodinating intermediate has not yet been elucidated. MAYBERRY (1964) has presented good arguments suggesting that it is molecular iodine (I_2). Others, however, have claimed that kinetic data are more compatible with a mechanism involving a free iodine radical ($I^{\cdot}$) (NUNEZ, 1969). Finally, hypoiodous acid (HIO^+) or iodinium ion (I^+) are also possible intermediates (HARINGTON, 1944). Whatever the intermediate is, it must be extremely short-lived, since the only forms of iodine found in the thyroid are organic iodine or iodide. Although in chemical systems organic iodination with oxidized iodine intermediates takes place spontaneously, there is now little doubt that the process is catalyzed enzymatically in the thyroid gland. The enzyme involved is most probably a heme-containing peroxidase which has recently been isolated in several laboratories (COVAL, 1967; TAUROG, 1970; DANNER, 1971; POMMIER, 1972). The enzyme is firmly bound to particles which sediment with the microsomal fraction, and relatively drastic procedures are necessary to render it soluble. The various procedures used by different investigators for solubilization probably account for the considerable variations in the chemical properties (molecular weight, heme content, k_m, etc.) from one laboratory to the other. The enzyme uses iodide and hydrogen peroxide as substrates, the latter being generated, for example, during the oxidation of flavin nucleotides. The enzyme may catalyze both the oxidation of iodine, and the transfer of the oxidized intermediate onto thyroglobulin (NUNEZ, 1969; POMMIER, 1973; see p. 283).

c) Coupling Reaction (step 3, Fig. 3)

In theory, thyroxine could be synthesized by the tetraiodination of thyronine, but this pathway is very unlikely, mainly because thyronine has never been found in thyroid tissue. Since v. MUTZENBECHER (1939) had shown that under slightly alcaline conditions diiodotyrosine condenses spontaneously to yield an appreciable amount of thyroxine, this has been the generally accepted pathway of thyroxine biosynthesis (HARRINGTON, 1944; DEGROOT, 1965). The coupling may proceed by the phenoxide ion of one diidotyrosine attacking ring carbon number 1 of a quinoid intermediate of the other diidotyrosyl residue, as envisaged by HARINGTON (1944). Very recently, LAMAS (1972) has reported good evidence that this coupling reaction is also catalyzed by the above-mentioned heme-peroxidase. Since both the subtrate (diiodotyrosine) and the product (thyroxine) of the reaction are part of the thyroglobulin molecule, it seems likely that the reaction involves two peptide-bound diiodotyrosyl residues. However, other reaction schemes involving one peptide-bound diiodotyrosyl and one free derivative, probably diiodohydroxyphenylpyruvic acid, also seem possible (MELTZER, 1961; KOJ TOI, 1965; BLASI, 1969; OGAWARA, 1972). While thyroxine arises from the coupling of two diiodotyrosine molecules, triiodothyronine can be synthesized by the coupling of one monoiodotyrosine to one diiodotyrosine, but this appears to be a minor pathway of triiodothyronine biosynthesis. Recent investigations have shown that the normal human thyroid gland secretes very little triiodothyronine. A large part of this hormone is produced peripherally by the monodeiodination of thyroxine (see p. 143).

d) Thyroglobulin and the Significance of Hormone Stores

Thyroglobulin obviously occupies a central place in the biosynthetic pathway of thyroid hormones, since both the iodination of tyrosyl residues and the coupling reaction proceed on this protein. It has a molecular weight of 660000 and a sedimentation coefficient of 19 S. A small amount of closely related protein with a sedimentation coefficient of 27 S, possibly a dimer of the 19 S protein, is always demonstrable in normal glands. Thyroglobulin contains about 10% carbohydrate. The amino acid and carbohydrate composition of human and other thyroglobulins has been analyzed in great detail (SPIRO, 1970; ARIMA, 1972).

The subunit structure of thyroglobulin is complex and not yet fully elucidated. In an alkaline medium and after reduction of the disulfide bonds thyroglobulin dissociates into 4 subunits of 165000 molecular weight (EDELHOCH, 1965; DE CROMBRUGGHE, 1966). Whether all the subunits are similar or whether the molecule is asymmetric (LISSITZKY, 1968) is still a subject of debate, and much smaller subunits have recently been described (ROLLAND, 1972). The peptide subunits are assembled separately on the ribosomes; the carbohydrate units are added in the endoplasmic reticulum (SPIRO, 1966, 1969) and in a last step the fully assembled protein is iodinated (SEED, 1965; NUNEZ, 1965; VECCHIO, 1972). Normal human thyroglobulin has an iodine content of about 0.2 to 0.7%. Each molecule contains about 120 tyrosyl, 5 monoiodotyrosyl, 3 diiodotyrosyl and 1 to 2 thyroxyl residues. The average triiodothyronine content is less than one residue per mole (ROLLAND, 1972). It must be stressed here that the thyroid

hormones are *covalently* linked to thyroglobulin by peptide bonds, in contrast to their state in plasma, where they are reversibly associated with certain proteins by *non-covalent* bonds (see below).

Thyroglobulin is secreted into the peripheral blood, possibly via lymphatics, in only minimal amounts, except in certain inflammatory and hyperfunctional states of the gland, where significant serum concentrations have been measured (TORRIGIANI, 1969; VAN HERLE, 1973). A small amount of iodinated protein unrelated to thyroglobulin is also present in the normal thyroid. This protein shares many properties with serum albumin (ROBBINS, 1959). It is probably synthesized in the thyroid gland itself (OTTEN, 1971).

The thyroid gland contains remarkably large stores of hormone. The normal iodine content of the gland is about 10 to 20 mg, 30% of which are contained in thyroxine. With an average thyroxine iodine secretion of 50 µg/day (see below) the thyroxine stores would thus be enough to maintain euthyroidism for 50 to 120 days in theory. These large hormone stores are of practical importance, since they explain why antithyroid drugs which block hormone synthesis do not have an immediate effect on thyroid hormone secretion.

e) Hormone Release, Hormone Secretory Rate

Thyroxine and triiodothyronine are stored in the colloid as peptide-linked amino acids of thyroglobulin. When hormone is to be released, small droplets of colloid are engulfed by streamers growing from the apical cell membrane (step 4, Fig. 3). The droplets move into the cell by a process called endocytosis. The colloid droplets then move toward the basal cell pole, fusing en route with primary lysosomes. These particles are thought to contain the proteolytic enzymes necessary for the hydrolysis of thyroglobulin (step 5, Fig. 3). The thyroxine and triiodothyronine thus liberated diffuse into the blood, while mono- and diiodotyrosine are immediately deiodinated within the gland (step 6, Fig. 3) by a potent and quite specific deiodinase (DUMAS, 1973). A large part of the iodide arising is reincorporated into thyroglobulin. It is still matter of discussion whether trace amounts of iodotyrosines leak out from the thyroid. Mono- and diidotyrosine have been identified in peripheral blood in concentrations of about 1 µg/100 ml (WEINER, 1967). More recently radioimmunoassay has yielded a serum concentration of about 250 ng/100 ml for diidotyrosine (NELSON, 1972). The source of these plasma iodotyrosines is not known and it is not at all certain that it is the thyroid.

In the past few years it has been established beyond all doubt that the thyroid gland also secretes iodine in a nonhormonal form. In the dog this has been identified as iodide (NAGATAKI, 1969) generated within the thyroid gland by the deiodination of mono- and diiodotyrosine. There is now convincing evidence that a "nonhormonal iodine leak" of this kind also exists in the normal human gland (WARTOFSKY, 1971; BÜRGI, 1973).

The process of hormone release can be inhibited by pharmacological doses of iodide. The biochemical reaction underlying this iodide effect is totally unknown. The effects of several pharmacological agents on thyroid hormone release have been studied in an *in-vitro* system by WILLIAMS and WOLFF (1971). Colchicine is a potent inhibitor of thyroid hormone release in this system, which suggests that the microtubular-microfilamentous system is involved in the process. A short review on thyroid hormone release has been written by DEISS (1968).

TSH is the main controlling factor for hormone release. In the absence of TSH, hormone release remains minimal. One of the first effects of an injection of TSH on the thyroid is stimulation of endocytosis leading to an increase of colloid droplets within 1 min. Secretion of hormone into the blood follows rapidly (WILLIAMS, 1972).

The mean daily secretory rate for thyroxine in younger persons is about 80 µg per day, corresponding to 52 µg of iodine (ODDIE, 1966). GREGERMAN (1962) has shown that the thyroxine secretory rate declines with age from 80 µg per day at age 20 to about 45 µg per day at age 80.

Due to methodological difficulties the production rates of triiodothyronine are less well established. The best estimates available vary from 20 to 60 µg per day, corresponding to 12–36 µg of iodine (NICOLOFF, 1972; ODDIE, 1971; WOEBER, 1970). As outlined on p. 143 more than 50% of this is now thought to arise outside the thyroid from peripheral deiodination of thyroxine.

3. State of Thyroid Hormone in Plasma, and Disorders of Hormone Binding Plasma Proteins

The concentration of thyroxine in serum of normal persons varies between 5 and 11 µg per 100 ml (3.3 to 7.2 µg iodine per 100 ml). The normal value for triiodothyronine varies widely according to the method of measurement used. STERLING (1969) originally used competitive protein binding and obtained a mean value of

about 220 ng/100 ml. More recent studies using radioimmunoassays (ODDIE, 1971; MITSUMA, 1971; LIEBLICH, 1972; MCCONNON, 1971) or chemical iodine measurement (HORN, 1972) have yielded substantially lower values of 100–150 ng/100 ml serum.

In plasma, 99.97% of thyroxine and 99.70% of triidothyronine circulate bound to proteins. The bonds between protein and hormone are non-covalent, in contrast to the covalent bonds of the hormones to thyroglobulin within the thyroid. This means that the small free hormone fraction in plasma is in dynamic equilibrium with the protein-bound hormone and that ^{131}I-labeled hormone added to plasma rapidly binds to the proteins. Three proteins are involved in the binding: 1. thyroxine-binding globulin (TBG), an alpha globulin, which under specified experimental conditions binds about 60% of the circulating thyroxine. Its molecular weight is 58000*. At full saturation it can bind approximately 20 µg of thyroxine per 100 ml plasma; 2. thyroxine-binding prealbumin (TBPA), with which about 30% of the circulating thyroxine is associated. At full saturation it can bind up to 200 µg of thyroxine per 100 ml plasma; 3. albumin, which binds about 10% of the thyroxine and has a capacity of over 1000 µg per 100 ml plasma. New measurements taken in pH conditions closer to the *in-vivo* situation have shown that TBG probably binds more and TBPA less of the circulating thyroxine (WOEBER, 1968). Triiodothyronine binds less strongly to TBG than thyroxine and probably not at all, or only to a limited extent to TBPA (DAVIS, 1972). The theoretic and clinical implications of the reversible hormone binding to plasma proteins have been reviewed by OPPENHEIMER (1968) and amply discussed in Chap. I of this book (p. 4).

Most authors have found that the thyroid functional status correlates much more closely with the tiny fraction of free hormone than with the protein-bound or total plasma hormone (ROBBINS, 1967). Unfortunately, only specialized research laboratories can measure the free hormone, and most clinicians have to rely on measurements of the concentration of the total hormone. As has been outlined in Chap. I, in accordance with the law of mass action, alterations of serum proteins may elevate or lower the total plasma hormone concentration with no change in the functionally relevant free hormone level. Thus estrogens, now widely used in oral contraceptives, increase the concentration of TBG and cause misleadingly high values of the protein-bound iodine or the total serum thyroxine. The same situation is found in pregnancy, in porphyria (HOLLANDER, 1967), and in infectious hepatitis (VANOTTI, 1959). A rare hereditary disorder with high TBG levels has also been reported (BEIERWALTES, 1959; FLORSHEIM, 1962; REFETOFF, 1972; HODGSON, 1972), sometimes associated with goiter (SHANE, 1971). The reverse is true in patients treated with diphenylhydantoin, a drug which displaces thyroxine from its TBG binding sites and leads to low values for the total serum thyroxine (LARSEN, 1970). TBG levels are also lowered by high doses of corticosteroids and androgenic and anabolic steroids. Salicylates in high doses displace thyroxine from TBG and TBPA and lower the serum total thyroxine concentration (LARSEN, 1972). Stress and fever appear to lower the concentration of TBPA. During acute infections this may temporarily produce decreased binding of hormone to plasma proteins and a reciprocal increase of hormone binding to tissues (DE RUBERTIS, 1972). Androgenic steroids increase the concentration of TBPA. An hereditary decrease in the concentration of TBG has been described in several kindreds. When properly looked for, the defect is not so rare. Affected persons are usually detected incidentally. They have a low total serum thyroxine, but are euthyroid by all other criteria. The defect may be transmitted as an autosomal dominant (HEINONEN, 1970) or more frequently as an X chromosome-linked recessive (TORKINGTON, 1970; NUSYNOWITZ, 1971; REFETOFF, 1972). In the newborn and in the aged more thyroxine is bound to TBG and less to TBPA (SCAZZIGA, 1966). The significance of this finding is not clear. It certainly does not explain the decreased turnover of thyroxine in the elderly (BRAVERMAN, 1966).

4. Peripheral Hormone Metabolism, Conversion of Thyroxine to Triiodothyronine

The half-life of thyroxine in serum is 7 days, that of triiodothyronine 1 day (NICOLOFF, 1972), the difference probably reflecting the different proportion of serum hormone bound to proteins. The half-life of thyroxine is increased in hypothyroidism and decreased in hyperthyroidism. The distribution space of thyroxine is approximately 10 liters, that of triiodothyronine 43 liters (WOEBER, 1970). The liver contains one third of the total extrathyroidal thyroxine, which can rapidly exchange with the plasma thyroxine. Phenobarbital increases hepatocellular binding of thyroxine and stimulates the

* A much lower value has recently been reported (STERLING, 1971).

metabolic breakdown and the turnover rate of thyroxine, but does not alter the plasma concentration of free or protein-bound hormone (OPPENHEIMER, 1968). Only traces of the hormones can be found in the cerebrospinal fluid (CSF).

The thyroid hormones are inactivated mainly in the liver, kidney, brain, and muscle. A good review on thyroid hormone breakdown is given in a paper by PITTMAN (1973). Four processes are responsible for thyroid hormone metabolism:

1. Conjugation of the phenolic hydroxyl group with glucuronic or sulfuric acid. The conjugates are mainly excreted in the bile and a large proportion can be recovered in the feces, although most of the iodine atoms have been removed by deiodination, as discussed below. The ether linkage between the two thyroxine rings appears to remain largely intact during catabolism (PITTMAN, 1970).

2. Oxidative deamination and decarboxylation of the alanine side chain, processes taking place in the liver and in the kidneys. A relatively specific triiodothyronine aminotransferase has recently been demonstrated in these tissues (SOFFER, 1973).

3. Deiodination by a dehalogenase which is different from the enzyme which deiodinates iodotyrosines. This process is very active and has been extensively studied. When labeled thyroxine is given by injection, 80 to 90% of the iodine is excreted in the urine as iodide and the remainder appears in the feces (INGBAR, 1955). Deiodination of thyroxine takes place in muscle, liver, and kidney tissue. In liver part of the iodide is incorporated into butanol-insoluble, probably protein-bound compounds (KOZYREFF, 1970). The products of processes (2) and (3), thyronine and thyroacetic acid, have been unequivocally identified in human urine (PITTMAN, 1972).

An obligatory intermediate in the total deiodination of thyroxine appear to be the monodeiodinated derivatives. Since the deiodination occurs random in the α- or β-ring of the thyroxine molecule, these monodeiodinated intermediates are either the hormone 3,5,3'-triiodothyronine or the hormonally inactive 3,3',5'-triiodothyronine. Half of the degraded thyroxine may thus theoretically be converted to hormonally active triiodothyronine. Only recently has this pathway of triiodothyronine biosynthesis been recognized (STERLING, 1970; PITTMAN, 1971; BRAVERMAN, 1970; SCHWARTZ, 1971; SURKS, 1973). Measurements in man have shown that between 30 and 45% of the degraded thyroxine yields triiodothyronine. This accounts for at least half, if not all of the triiodothyronine produced in the body. The fact that most of the triiodothyronine is produced in extrathyroidal tissues fits well with recent observations that the normal human thyroid gland contains very little triiodothyronine (CHOPRA, 1973). SCHWARTZ (1971) has put forward the daring hypothesis that thyroxine is inactive per se and needs to be converted to triiodothyronine to exert its hormonal effects. Propylthiouracil inhibits the peripheral conversion of thyroxine to triiodothyronine (OPPENHEIMER, 1972, p. 266). Whether this effect is relevant to its antithyroid action in clinical use remains to be established.

4. About 8 μg thyroxine and 3 μg triiodothyronine are excreted unchanged in the urine. This pathway has only recently been explored and offers great promise as a diagnostic test for thyroid function (CHAN, 1972; BURKE, 1972).

5. Mode of Action of Thyroid Hormones

Both clinically and biochemically, thyroid hormones display a confusing multitude of effects on the most varied tissues. Despite considerable research efforts no common denominator or simple biochemical mechanism has yet been detected that could explain all thyroid hormone effects by a unified concept. It now appears that in order to understand thyroid hormone action it is necessary to consider processes as varied as its effects on the cell nucleus, on RNA-synthesis and on cell differentiation, its stimulation of protein synthesis at the translational (ribosomal) level, its effects on mitochondrial respiration and protein synthesis, and finally its influence on the adenyl cyclase system of certain cell membranes individually.

Thyroid hormones bind to many components of peripheral tissues, e.g. to mitochondria in the liver (HOCH, 1968), to soluble cytosol proteins of the kidney (STERLING, 1972), and in the case of triiodothyronine, to nuclei of kidney and liver cells (GRISWOLD, 1972; OPPENHEIMER, 1972). Specific binding sites are present in the anterior pituitary for triiodothyronine but not for thyroxine (SCHADLOW, 1972).

In GRISWOLD'S (1972) experiments, thyroxine injected into tadpoles bound rapidly and firmly to the chromatin of liver cell nuclei. Chemically the bound hormone appeared unaltered. If the tadpoles were kept at 4°C the binding did not take place, which is compatible with the finding that its action on enzyme induction is also inhibited by low temperature. Whether the transfer of thyroxine to the nucleus is similar to that of steroid hormones (p. 15), requiring a soluble cytoplasmic receptor, remains to be established.

The binding sites for thyroid hormone have so far not been further characterized and it is doubtful whether they are all true hormone receptors. Most of the studies have been performed with isotopically labeled thyroid hormones and in some instances it was not easy to remove bound radioactivity from the alleged receptors, as would be expected if the hormone-receptor interaction was reversible, as is postulated by the general theory of hormone action.

Thyroid hormones are also metabolized by many of the tissues they affect (TATA, 1961; MORREALE DE ESCOBAR, 1967, see p. 266; see also preceeding section). OPPENHEIMER (1971) has studied the relationship of thyroid hormone metabolism to thyroid hormone action. Increased hepatic thyroxine metabolism induced by phenobarbital in his experiments was not associated with an increased hormone effect, and he concluded that hormone breakdown was not essential for hormone effect. GALTON (1962), however, has demonstrated that thyroxine has no calorigenic effect in poikilothermic animals which do not metabolize the hormone. The provocative view that thyroxine has no effect *per se,* but must be converted peripherally to thyroxine to be active (SCHWARTZ, 1971) is still a matter of considerable debate. The latest review on all aspects of thyroid hormone action dates back to 1962 (HOCH).

a) Effects on Mitochondria and Respiration

The most conspicuous action of thyroid hormones is their ability to increase oxygen consumption and heat production. This effect is demonstrable in liver, kidney and muscle, but not in the gonads, genital organs, brain, and lymphatic tissue (BARKER, 1952). The ineffectiveness in the latter tissues cannot be attributed to a lack of penetration of hormone, since labeled thyroxine can be recovered from these tissues after injection.

The cellular sites of respiration are the mitochondria, small organelles with a rather complex structure and biochemistry (TAPLEY, 1967). In connection with thyroid hormone action, two fundamental properties of oxidation in mitochondria must be briefly recapitulated. The first is that normally for each atom of oxygen consumed the mitochondrion produces three high energy phosphate bonds as ATP, leading to a so-called P/0 ratio of about three. This phenomenon is known as "coupling" of respiration. The second property of mitochondrial respiration is that of "control" by the amount of substrate available, such as ADP. This control can be demonstrated *in vitro* by the fact that in the absence of ADP (so-called state 4) mitochondrial oxygen consumption is substantially lower than in its presence (state 3). The ratio of state 3 to state 4 respiration is called the "respiratory control ratio".

Ever since the demonstration that thyroid hormone *in vitro* uncouples oxidation, i.e. decreases the number of high-energy phosphate bonds in proportion to the oxygen consumed (HOCH, 1954; LARDY, 1954; MARTIUS, 1955), it has been argued that this constituties its basic mode of action. However, there are several observations which are hard to reconcile with this view. Firstly, high, unphysiologic concentrations of thyroxine are necessary to uncouple respiration. Secondly, D-thyroxine, which has only a low activity *in vivo*, is as effective an uncoupling agent as L-thyroxine. Thirdly, dinitrophenol, a potent uncoupling agent, though increasing oxygen consumption, does not reverse the other symptoms in hypothyroid patients. Lastly, treatment of thyroidectomized animals with thyroxine does not decrease the P/0 ratio (BRONK, 1962). Although there has been one report of uncoupled respiration in mitochondria from hyperthyroid patients (ERNSTER, 1959), this has not been confirmed in later careful studies (STOCKER, 1968). Stocker also found a normal respiratory control ratio in the mitochondria of hyperthyroid patients, which is in contrast to animal experiments, where thyroxine given *in vivo* lowers the respiratory control (HOCH, 1967; VOLFIN, 1969; KIMATA, 1971). The current prevailing view is that thyroid hormone undoubtedly increases oxygen consumption, and may depress the respiratory control ratio, but that it does not cause uncoupling in concentrations that occur in physiological conditions or in hyperthyroidism.

More recently it has been recognized that thyroid hormone stimulates protein synthesis in mitochondria. This effect occurs within 3 hours of a thyroxine injection, i.e. much earlier than the stimulation of ribosomal protein synthesis discussed below (PRIMACK, 1970). VOLFIN (1969) found that thyroxine specifically stimulated the synthesis of a few distinct mitochondrial proteins. The relationship between this early protein synthesis and the stimulation of respiration is not clear. KAPLAY (1971) reported that he eluted a protein from thyroxine-stimulated mitochondria which, when added *in vitro* to untreated mitochondria, enhanced their oxygen consumption. BUCHANAN (1971), in contrast, found that thyroxine stimulated oxygen consumption in mitochondria even when mitochondrial protein synthesis was blocked with chloramphenicol or puromycin.

Finally it has been observed that thyroxine produces swelling of mitochondria *in vitro* (LEHNINGER, 1959, 1962; GREIF, 1962). This effect is not specific and can be elicited with insulin. Its significance is unknown. ISMAIL-BEIGI (1971) has related the calorigenic effect of thyroid hormone to the transport of Na^+ or K^+ across the cell membrane. He found that thyroid hormone did not stimulate oxygen consumption in liver tissue when the Na^+ or K^+ transport was inhibited by ouabain. He proposed that the primary effect of thyroid hormone was the stimulation of Na^+ transport, which in turn necessitated a higher oxygen consumption to provide the required energy.

b) Effects on Cellular Differentiation, Growth, and Protein Synthesis

The effects discussed under this heading are all interrelated biochemically and are therefore best treated together. Probably one of the most spectacular hormone effects known to biologists is the induction of the metamorphosis of tadpoles by thyroxine. Indeed it seems that the only function of thyroid hormone in anurae is to assure proper metamorphosis. The study of this phenomenon has come a long way from the classic morphologic and embryologic investigations towards a biochemical understanding of the processes involved. An excellent example is provided by the work of COHEN and co-workers (PAIK, 1961; COHEN, 1970), who studied the appearance of the enzymes necessary for urea synthesis in the liver. These enzymes are not present in the tadpole. They have provided conclusive evidence that thyroxine is necessary for this enzyme induction and that its site of action is the cell nucleus, where it stimulates the synthesis of DNA-dependent RNA polymerase, an enzyme necessary for the production of mRNA (GRISWOLD, 1972, see Chap. I).

The fact that hypothyroid children have a decreased growth rate which is restored to normal by thyroid hormone treatment has its biochemical correlate in the stimulatory effect of thyroxine on protein synthesis. Within 2 hours after administration of triiodothyronine to animals by injection, the microsomal protein synthesis in the liver is enhanced (SOKOLOFF, 1968). This "early" effect on protein synthesis is dependent upon the presence of mitochondria or an ATP-generating system in the incubation medium (CARTER, 1971). It should not be confused with the rapid stimulation of mitochondrial protein synthesis described in the previous section, since it is clearly an effect on ribosomal function. The effect is independent of mRNA synthesis; it precedes any change in mRNA concentration in the cell and it is not blocked by actinomycin D. Thus it must be a hormone action at the translational (ribosomal) level (see also Chap. I). An independent "late" effect on protein synthesis in the liver can also be observed (SOKOLOFF, 1964; TATA, 1966, 1967, 1968). This late effect is independent of the presence of mitochondria, but requires the synthesis of mRNA. It is therefore thought to be due to a hormone effect on the transcription of RNA in the cell nucleus, analogous to changes observed during anuran metamorphosis. Thyroid hormone effects on the enzymes of the central nervous system are discussed on p. 147.

c) Effects on the Cardiovascular System and on the Adenyl Cyclase System

It is a familar clinical observation that thyroid hormone increases the heart rate and widens the pulse pressure. In hyperthyroidism the stroke volume usually increases but occasionally remains constant or decreases. The cardiac output increases, and, although the peripheral vascular resistance decreases, the mycocardial workload becomes greater. The cardiac output increases more than would be expected on the basis of the enhanced peripheral oxygen consumption (DE GROOT, 1970). Increased excitability of the cardiac conductive tissue is found before a change in heart rate (ZAIMIS, 1969). Premature beats and auricular fibrillation occur frequently in hyperthyroidism (cf. p. 184, 207).

The effects of thyroid hormone on the heart are reversed in part by beta-adrenergic blocking agents (see p. 150 and p. 197). In many ways the effects of thyroid hormones on the heart resemble those of catecholamines. It is now firmly established that the catecholamine actions on the heart are mediated by the adenyl cyclase system (Chap. I), and it therefore seemed logical to look for a similar mechanism in the case of thyroid hormones. The first hypothesis to be examined was whether thyroid hormone simply acted by increasing the myocardial sensitivity to catecholamines. This view was clearly refuted by observations that thyroid hormone affected the contractility regardless of the level of circulating catecholamines (BUCCINO, 1967) and that the dose-response curve for epinephrine on papillary muscles of hyperthyroid cats was identical to that obtained with tissues from normal cats, both when cyclic AMP generation was measured and when contractility was used as a measure of response (LEVEY, 1969). A second question was whether the amount of adenyl cyclase was controlled

by thyroid hormone. Although myocardial total adenyl cyclase was slightly diminished in hypothyroidism, this was not thought sufficient to account for the effects of thyroid hormones (LEVEY, 1971). Most evidence now points to a direct stimulation of adenyl cyclase by the thyroid hormones independent of that of catecholamines (LEVEY, 1969). This complex problem has recently been well reviewed by LEVEY (1971), but his view has been challenged by recent work of WILDENTHAL (1972), who maintained fetal mouse hearts in organ culture and was therefore able to study the effects of thyroid hormone in carefully controlled catecholamine concentrations. Exposure of the explanted hearts to triiodothyronine for three days did not change their basal beating rate, but sensitivity to norepinephrine was significantly enhanced. Further research is necessary on this difficult question.

d) Effects on Lipid and Adipose Tissue Metabolism

Thyroid hormones enhance the degradation of cholesterol, and to a lesser degree its synthesis. As a result, serum cholesterol is low in hyperthyroidism and high in hypothyroidism. It has also been known for some time that thyroid hormone increases lipolysis and the release of free fatty acids in adipose tissue (CHALLONER, 1970). KRISHNA (1968) has suggested that this effect is due to an increased amount of adenyl cyclase by *de-novo* synthesis of enzyme protein. a view corroborated by CALDWELL'S (1971) findings.

e) Thyroid Hormone Effects on the Central Nervous System and on Neuromuscular Function

In view of the profound neurologic changes observed in hyper- and hypothyroidism there can be no doubt that thyroid hormone affects brain and nerve metabolism. Hypothyroidism produces a general slowing of most cerebral functions, although orientation in space and time and recognition of objects are maintained intact except in the severest cases. The biochemical substrate for this phenomenon is not known. Since thyroid hormones have no influence on cerebral O_2 consumption (BARKER, 1952) it cannot be explained by decreased energy metabolism. It may be related to the stimulatory effect of thyroid hormone on cerebral protein biosynthesis (GELBER, 1964). In adult men or animals, hypothyroid changes in cerebral function are entirely reversible with thyroid hormone substitution. This is not true of cerebral changes produced by hypothyroidism in fetal life or during infancy. It is a common clinical observation that congenitally hypothyroid children remain mentally retarded despite thyroid hormone substitution if there has been any delay in instituting the treatment. Recent advances in neurobiochemistry have provided detailed information on the development of the central nervous system. It appears that cerebral maturation proceeds in a series of steps with the synthesis of particular proteins, enzymes, and lipids, each appearing at a relatively fixed point in fetal or infantile life. In rats, WYSOCKI (1972) has studied the appearance of two enzymes associated with myelin formation in the nervous system, UDP galactose: sphingosine galactosyl transferase, and 2′:3′ nucleotide 3′-phosphohydrolase. Both enzymes are present in reduced amounts in brains of hypothyroid neonate rats. Regular thyroid hormone substitution from the first day of life onward brings the enzyme back to normal. If thyroid hormone substitution is not started until the 8th day of life the enzymes remain irreversibly low. Unfortunately, comparable information is not available for man and we do not known the exact time when thyroid hormone lack produces irreversible brain damage.

Muscular contraction is impaired in hypo- as well as in hyperthyroidism. An extreme form is observed in Japan where there is a strikingly high incidence of acute attacks of hypokalemia and muscular paralysis (p. 186). In a careful study of thyroid hormone effects on the neuromuscular junction, HOFMANN (1972) showed that the membrane potentials of hyperthyroid muscles were decreased. This may explain the susceptibility of hyperthyroid patients to hypokalemic paralysis. Very few electrophysiological changes were observed in hypothyroid nerve-muscle preparations, and no explanation was found for the clinically important slowing of reflexes in hypothyroidism.

f) Other Thyroid Hormone Effects

Thyroid hormone increases calcium and phosphate turnover and calcium loss in both stools and urine. Osteoblasts and, even more, osteoclasts become activated in hyperthyroidism, producing a measureable increase of the alkaline phosphatase of serum (EPSTEIN, 1968). Hyperthyroidism is also often associated with osteoporosis. However, hypothyroid patients tend to become hypercalcemic easily under calcium loads. Patients who suffer from hypoparathyroidism and hypothyroidism after thyroid sugery usually show a decreased requirement for vitamin D when properly substituted with thyroxine. Thyroxine treatment markedly enhances bidirectional transport (in particular

influx) of magnesium and calcium in liver slices (WALLACH, 1972).

Thyroid hormone has a complex effect on carbohydrate metabolism. In high doses thyroxine diminishes the liver glycogen content, while in small doses it enhances glycogen synthesis. Oral glucose tolerance tests are sometimes abnormal in hyperthyroidism. This may be due partly to a more rapid intestinal sugar absorption and partly to increased gluconeogenesis (HEITZMANN, 1971). Thyroid hormone probably enhances glucose metabolism in adipose tissue (BUTTERFIELD, 1964). Hyperthyroidism increases the caloric and therefore the insulin requirement in concomitant diabetes.

During hyperthyroidism, urinary creatine excretion is increased and creatinine excretion decreased. The reverse is true in hypothyroidism. The synthesis of creatine is probably not changed, but creatine cannot be retained in the tissues in hyperthyroidism due to inhibition of creatine phosphokinase (KUHLBÄCK, 1957; ASKONAS, 1959).

Thyroid hormones produce a proliferation of lymphatic tissue. The thymus is often atrophic in hypothyroidism and enlarged in hyperthyroidism, but this may also be due to associated autoimmunologic processes.

Thyroid hormone stimulates hemoglobin synthesis *in vitro* in bone-marrow cultures (FUHR, 1970). This may have some connection with the anemia often present in hypothyroidism, but doctors will object that moderate unexplained anemia is also a feature of hyperthyroidism. Thyroid hormone added to erythrocytes *in vitro* increases their content of 2,3-di-phosphoglyceric acid by an activation of 1,3-di-phosphoglyceric acid mutase. Since the former compound has a profound influence on oxygen transport in blood, this effect may have some clinical importance (SNYDER, 1970).

6. Control of Thyroid Hormone Biosynthesis and Release

The main factor controlling thyroid function is TSH. In addition, the thyroid has the capacity for limited autonomous control.

a) Hypothalamic Control, TRH

It was recognized long ago that neural factors were involved in the control of TSH secretion in laboratory animals. For example, a low ambient temperature, stress of any other kind and a change in atmospheric pressure* were recognized as being capable of increasing thyroid hormone secretion under appropriate conditions. After small lesions at specific sites of the hypothalamus, rats showed signs of hypothyroidism and a lowered serum thyroid hormone concentration, though the TSH feedback was not completely abolished and the animals were still capable of responding to a goitrogen with thyroid hyperplasia. It is from these observations that the concept of the neuroendocrine control of TSH secretion has evolved (BROWN-GRANT, 1957, 1960, 1967; AVERILL, 1961; GUILLEMIN, 1964). Further studies have culminated in the isolation of a tripeptide from porcine (NAIR, 1970) or ovine (BURGUS, 1970) hypothalamus, which after injection into animals or man or after addition to the incubation medium of surviving pituitary slices causes the release of TSH. The chemical formula of this tripeptide, which has been termed thyrotropin-releasing hormone (TRH), is L-(pyro)glutamyl-L-histidyl-prolinamide. The hormone can now be chemically synthesized in large quantities and the easy availability has led to numerous publications on its effects and the development of a very useful clinical test (ANDERSON, 1971). Short reviews have been published by BURGUS (1970) an HERSHMAN (1971).

The main features of the hypothalamic control of TSH secretion are as follows: Upon neural inputs, which can also be generated by implanted electrodes, the hypothalamus secretes TRH into the venous blood, where its concentration can be measured by bioassay (WILBER, 1970). Before being diluted in the total circulating blood volume TRH reaches the anterior pituitary via a small system of portal vessels and produces a rapid release of TSH. Feedback control by thyroid hormone probably operates on several loops of this complex system. One loop, probably the main one, functions by a direct effect of thyroid hormone on the anterior pituitary. This is nicely demonstrated by the fact that TSH secretion is greatly increased upon injection of a standardized amount of TRH in hypothyroid patients and decreased or absent in hyperthyroid patients. In addition, receptors for triiodothyronine have recently been observed in the pituitary (SCHADLOW, 1972, p. 251). A second feedback loop goes to the hypothalamus, where thyroid hormone inhibits the release of TRH, but at present this loop is more difficult to demonstrate experimentally. The first loop (thyroid-pituitary) can operate in the absence of TRH, although TSH secretion is diminished. In other words, TRH changes the set point of the pituitary response to the circulating thyroid hormone level.

* It must be emphasized that in man the same stimuli provoke no or only a limited response of TSH secretion.

b) Anterior Pituitary. TSH

The function of the normal thyroid gland is controlled by thyrotropin (thyroid stimulating hormone, TSH), which is produced by the basophil cells of the anterior pituitary gland. TSH for diagnostic and research purposes is usually prepared from pituitary extracts. Highly purified preparations have been made from such extracts, and LIAO (1971) has recently reported the primary structure of bovine TSH. The hormone contains 15% carbohydrate and consists of two peptide chains, one of which (the alpha chain) is identical with the alpha chain of luteinizing hormone (LH). Hybrids between TSH-beta and LH-alpha have normal biologic activity on the thyroid comparable to that of recombined TSH-alpha and TSH-beta (PIERCE, 1971). The molecular weight of the hormone is 28000. Even the purest preparations are found not to be homogeneous when tested by sensitive methods such as gel electrophoresis. This microheterogeneity is probably not the result of variations in the primary amino-acid sequence, but rather of a varying amide or carbohydrate content. The potency of TSH preparations is usually measured in a bioassay and expressed in units of an international or a comparable USP standard. More recently, a limited supply of a standard containing human TSH, so-called HTRS-A, has become available (see REICHERT, 1970, for comparison of both standards). The purest TSH preparations have potencies of 20–40 U/mg, exceptionally 100 U/mg (BATES, 1960 and 1968). Human and bovine TSH show incomplete immunologic cross-reactivity. In the mouse bioassay, human and bovine TSH give different standard curves.

TSH affects numerous parameters of the metabolism of the thyroid gland. Within minutes after injection or addition to an incubation medium, stimulation of colloid endocytosis, of glucose oxidation, of phosphate incorporation into lipids and of RNA synthesis are easily measured. The last effect is probably a consequence of increased ribose availability due to oxidation of glucose via the pentose monophosphate pathway, but the other effects seem to be independent of each other. TSH also enhances synthesis of thyroglobulin and incorporation of iodine into organic compounds. On the whole, TSH-induced colloid endocytosis predominates over thyroglobulin synthesis, and TSH-stimulated glands are characteristically poor in colloid and thyroglobulin. Active iodide transport is also stimulated by TSH, but this effect has proved more difficult to demonstrate, because it has a latency of several hours. Finally, TSH causes growth of the thyroid gland. Most, if not all, effects of TSH are mediated by the adenyl cyclase-cyclic AMP system and can be reproduced by the addition of cyclic AMP or derivatives (J. B. FIELD, 1968; BURKE, 1971). Cyclic AMP-dependent protein kinases have also been demonstrated in thyroid tissue (RAPPAPORT, 1971). Recent short (FIELD, 1968; BURKE, 1971; LIBERTI, 1971) and extensive (DUMONT, 1971) reviews on TSH action are available. An interesting feature of unknown physiologic significance is that TSH also has extrathyroidal effects. Notably it enhances lipolysis in adipose tissue (FREINKEL, 1961).

TSH can now be measured in human serum by a specific radioimmunoassay. Most assays use rabbit antisera against human TSH, rarely against bovine or porcine TSH. The human TSH research standard A is the reference source. Initially the assays were not sensitive enough to measure TSH in all normal subjects (ODELL, 1967). In the meantime, sensitivity has been improved and TSH is now dectectable in most, though still not all, sera of normal persons (HALL, 1971; PATEL, 1971). Normal values vary considerably from one laboratory to the other due to the fact that each research group uses its own antibody and sometimes also individual standards. The most sensitive assays now detect any values over 0.5 μU/ml (HALL, 1971, PATEL, 1971) and the normal range extends from unmeasurable to about 5 μU/ml. Values over 10 μU/ml are clearly above the normal range in most current assays. The reliability and clinical usefulness of the TSH radioimmunoassay are now well established (HERSHMAN, 1971; MAYBERRY, 1971), although problems of sensitivity and specificity still persist, particularly in the low and low normal range. It is difficult to understand, for example, that many laboratories measure normal values in sera of frankly thyrotoxic patients (BECKERS, 1971), when low or unmeasurable values would obviously be expected. In addition, bioassay measurements give consistently lower values in normal sera than the immunoassay (ADAMS, 1972). Technical improvements to the latter method have recently overcome some of the difficulties (PATEL, 1971).

The TSH distribution space is about 50% larger than the plasma volume. The serum half-life of injected TSH is 30–100 min in euthyroidism. It is longer in hypothyroidism and shorter in hyperthyroidism (ODELL, 1967). ODELL (1967) found a mean daily secretory rate of 165 mU, while BECKERS (1971) reported a value of 420 mU/day and attributed his higher value to the borderline low iodine intake of his normal subjects.

As anticipated, the plasma TSH concentration shows a close inverse correlation with the concentration of the free portion of serum thyroxine (REICHLIN, 1967), and very high values are found in primary hypothyroidism. In fact, the TSH immunoassay has been most useful in detecting borderline cases of thyroid failure, since TSH levels are often frankly elevated when the serum total thyroxine is still in the normal range.

In the first 24 hours after birth TSH in plasma shows a sharp rise to high levels and returns to normal after one or two days. The values then remain very constant throughout childhood and adult life. In rat diurnal variations of TSH secretion have been demonstrated by BAKKE (1965). Such variations were not detectable in man initially (ODELL, 1967; LEMARCHAND, 1969; WEBSTER, 1972), but NICOLOFF (1970) has recently obtained good evidence for a diurnal rhythm of thyroidal iodine release, which he attributes to oscillations in TSH secretion. From very recent measurements it appears that TSH levels are very constant throughout night and day except in the early morning hours, when a short marked peak is observed (VANHAELST, 1972). Unlike rats, humans do not react to exposure to cold with secretion of TSH (ODELL, 1967), except in children in whom severe hypothermia is induced for heart surgery (WILBER, 1970).

c) Thyroid Autoregulation

Evidence for thyroid autoregulation independent of TSH stems largely from experiments in hypophysectomized rats subjected to iodine deficiency, and little is known about its importance in man. Thus it has been found that the thyroid gland may control its hormone secretion in response to small changes in the plasma iodide level or the total thyroidal iodine content before changes in circulating TSH become apparent (STUDER, 1965, 1966; STEIGER, 1969). Hypophysectomized rats are still capable of increasing active iodide transport in response to iodine deficiency (HALMI, 1956), and it has been postulated that an iodinated organic iodine intermediate, if present in sufficient amounts, will depress active iodide transport. In iodine deficiency this organic iodine compound would be diminished and iodide transport activated. INGBAR (1972) has written a concise review on thyroid autoregulation.

d) Prostaglandins, Biogenic Amines and Autonomic Nervous System

Prostaglandin PGE-1 reproduces practically every effect of TSH on thyroid slices *in vitro* by activating adenyl cyclase (ZOR, 1969). In mice, epinephrine, norepinephrine, 5-hydroxitryptamine, and even electrical stimulation of the cervical sympathetic trunk cause colloid droplet formation (MELANDER, 1972). Vagal stimulation has also been shown to induce hormone release (ISHII, 1968). In isolated thyroid cells epinephrine and serotonin stimulate organic iodination and glucose metabolism (MAAYAN, 1968, 1971). The vascular blood supply of the thyroid also appears to be influenced by the autonomic nervous system (SÖDERBERG, 1959; LEAK, 1970). The physiologic importance of these effects is not yet known.

7. The Thyroid in Relation to Other Endocrine Glands

a) Adrenal Medulla

The effects of thyroid hormones, particularly on the heart, resemble those of catecholamines. Alpha-receptor blocking agents, such as phentolamine, may diminish the tachycardia, hyperhydrosis, and tremor of hyperthyroidism even while not affecting oxygen consumption and cardiac index. Beta-receptor blocking drugs also produce an impressive improvement of the tremor, tachycardia, and other symptoms of hyperthyroidism, including the increased cardiac index (HOWITT, 1966; PIETRAS, 1972) and reduced circulation time (GROSSMAN, 1971). This may be interpreted as indirect evidence that the thyroid hormones act via the catecholamines, e.g. by sensitizing the tissues to the effects of epinephrine. However, present experimental evidence (WILSON, 1966; ZAIMIS, 1969; see also section on mode of action of thyroid hormones) is not compatible with this view, and it is now generally accepted that thyroid hormones exert a catecholamine-like effect *per se*.

In some experimental conditions epinephrine may enhance the synthesis and release of thyroid hormones (FALCONER, 1965, 1967, see also preceding section). Norepinephrine increases thyroid blood flow and produces an increase in the volume of the gland, possibly by constriction of the small veins (MOWBRAY, 1960).

Epinephrine enhances the peripheral degradation of thyroid hormone in rat (GALTON, 1965), but in man the reverse may be true. The problems of thyroid-catecholamine interrelations have been reviewed by LEAK (1970).

b) Adrenal Cortex

Cortisol breakdown is enhanced in hyperthyroidism. Due to the feedback mechanism,

which leads to an increased secretion of ACTH and hypertrophy of the adrenal cortex, normal cortisol concentration in plasma is maintained. The excretion of 17-hydroxycorticoids is also increased. In hypothyroidism, the metabolism of the adrenal cortical hormones by hydrogenation is decreased, and the excretion of 17-hydroxycorticoids is reduced.

In severe hypothyroidism, the decreased metabolic clearance of cortisol leads to hypotrophy of the adrenal cortices via decreased ACTH secretion, with adrenal insufficiency in times of increased requirements. The response in the ACTH-test is variable, but the metopyrapon test reveals a normal ACTH reserve.

Cortisone in high doses inhibits the rate of secretion of the thyroid hormones, increases the renal iodine clearance, and diminishes the binding of thyroxine to thyroxine-binding globulin. On the other hand, in patients without adrenal glands, ACTH has a stimulating effect on the uptake of ^{131}I by the thyroid gland.

c) Ovaries

Estrogens increase the concentration of thyroxine-binding globulin in plasma. As outlined in the section on thyroid hormone transport, this produces a rise in the protein-bound iodine or the serum total thyroxine. However, the concentration of free thyroxine in plasma remains constant, and the euthyroid state is maintained. A resin T_3-uptake test in the hypothyroid range will alert the doctor to the fact that the increased serum total thyroxine reflects a change in thyroxine-binding globulin rather than hyperthyroidism. Hormonal contraceptives containing estrogens have the same effect.

d) Testes

Androgens and anabolic synthetic steroid hormones, in contrast to estrogens, lower the thyroxine-binding globulin. The serum total thyroxine becomes lowered, but the T_3-resin test changes in the opposite direction, assuming hyperthyroid values. The free serum thyroxine remains constant.

e) Thyroid Gland and Pregnancy

In pregnancy the thyroid gland may increase in size, but how often this occurs and whether it only takes place in areas of limited dietary iodine supply is still controversial. Due to the action of estrogen the total serum thyroxine rises and the resin T_3-uptake test is shifted into the hypothyroid range (see above). The free serum thyroxine again remains constant.

The basal metabolic rate increases during pregnancy. This is probably not due to a true hypermetabolic state, but reflects increased metabolic mass due to the fetal tissues, or increased cardiac work. The radioiodine uptake of the thyroid is moderately increased in pregnancy, but the significance of this finding is unknown (STEIN, 1973).

Fertility is much reduced in hypothyroidism. If pregnancy does occur, miscarriage or fetal malformations are frequent. Occasionally even a severely hypothyroid woman gives birth to a perfectly normal child. In contrast, mild to moderate hyperthyroidism has very few deleterious effects on pregnancy and the fetus, but this does not mean that the management of hyperthyroidism during pregnancy is easy, nor that it should be neglected. Children born to hyperthyroid mothers sometimes suffer from neonatal hyperthyroidism, allegedly due to placental transfer of LATS, which can be measured in the plasma of the offspring (p. 200). The condition clears spontaneously parallel to the disapperance of LATS from the plasma of the newborn.

TSH does not cross the placenta from mother to child or in the reverse direction. TSH-like substances can be extracted from human placental tissue, but their physiologic significance is totally unknown (see section on hyperthyroidism, p. 209).

The important question as to what extent thyroid hormones do cross the placenta has not yet been satisfactorily settled. In the human fetus the concentration of thyroid hormone in plasma is much lower than in maternal plasma, especially in the first two trimesters. However, fetal thyroxine-binding globulin is equally low, and therefore the crucial free thyroxine concentration could be the same on either side of the placental barrier in theory. More recently, careful measurements of free thyroxine in the maternal and fetal circulation from the 8th to the 24th week of gestation have established that before the 10th week no free thyroxine is present in the fetus. The concentration then rises rapidly, but there is no correlation with the maternal concentration (GREENBERG, 1970). When labeled thyroxine is admistered by injection to the mother, most of the ^{131}I is recovered in the fetus as iodide, and only a very small part as hormone. ROBIN (1972) has given an excellent short review of the problem. In his own experiments ^{131}I from labeled thyroxine injected into pregnant sheep appeared in the fetus mostly as iodide and only in small amounts as thyroxine. He concluded that there was a small but significant two-way flux of thyroxine across the placenta. Treatment of hyperthyroidism with

antithyroid drugs during pregnancy may produce fetal hypothyroidism and goiter despite the fact that the mother is intentionally left slightly hyperthyroid. In our view, all this evidence taken together confirms that thyroid hormones cross the placental barrier from mother to fetus to a very limited extent if at all, despite claims to the contrary (RAITI, 1967). The fetus is therefore dependent on its own production of thyroid hormone; since most antithyroid drugs, including iodide, cross the placental barrier, this greatly complicates the treatment of hyperthyroidism during pregnancy.

Iodide is actively transported across the placenta into the fetal circulation. This transport can be inhibited by thiocyanate (MYANT, 1958). Pharmacological doses of iodide administered to the mother, e.g. for the management of asthma, have produced severe fetal hypothyroidism and goiter (p. 167).

8. Changes of Thyroid Function in Diseases of Other Organs

a) Diseases of the Liver

In obstructive jaundice, conjugates of thyroxine, particularly glucuronides, circulate in the plasma and may elevate the level of protein-bound iodine (VANOTTI, 1959). Hepatitis and acute intermittent porphyria increase the thyroxine-binding globulin level and therefore the protein-bound iodine and the total serum thyroxine. In cirrhosis the thyroidal radioiodine uptake is sometimes increased, probably due to deficient dietary iodine intake associated with the poor nutritional status of alcoholics. The reverse, a low radioiodine uptake, has also been described in patients with cirrhosis (HOLLANDER, 1967). The variations of thyroxine-binding globulin in chronic liver disease are unpredictable, so that the total serum thyroxine may be normal, elevated or decreased (HOLLANDER, 1967). MCCONNON (1972) recently investigated the effect of liver cirrhosis on thyroid hormone turnover. He found that the production rate of triidothyronine was doubled in cirrhosis, whereas that of thyroxine was decreased by about 30%.

b) Renal Disease

In renal insufficiency iodide clearance by the kidney is decreased. Since an oral dose of radioactive iodine is divided between the thyroid gland and the kidney, the obvious consequence of renal disease might seem to be increased thyroidal uptake. This reasoning does not, however, take into account that the plasma iodide level rises due to impaired renal iodide clearance. This in turn lowers the thyroidal iodide clearance, either by autoregulation or by the TSH feedback system. Therefore thyroidal radioiodine uptake is in fact normal in most cases of kidney disease even in anephric patients (ODDIE, 1970). In the nephrotic syndrome the total serum thyroxine is often low. Whether this is due to a loss of hormone in the urine or to a loss or decreased synthesis of thyroxine-binding globulin (CRUCHAUD, 1954; RASMUSSEN, 1958) is not yet known. Usually the free thyroxine in the serum remains normal and a euthyroid state is maintained (HERMANN, 1972).

Increased amounts of TSH are excreted in the urine of rats rendered nephrotic by puromycin (IFF, 1969). Some clinical features of chronic uremia are similar to those of hypothyroidism, and FANKHAUSER (1968) has found some evidence of secondary hypothyroidism in about one third of uremic patients.

D. Hypothyroidism in the Adult

1. Classification

As with the other endocrine glands controlled by the anterior pituitary, hypofunction of the thyroid gland can be divided into a primary form due to failure of thyroid tissue itself and a secondary form due to a deficiency of TSH secretion. Plasma TSH is elevated in the former and low in the latter case. In primary hypothyroidism the thyroid gland is already under intense stimulation by endogenous TSH, and injection of TSH has no further stimulatory

Table 1. Thyroid diseases with hypothyroidism. The nomenclature, but not the classification, of the American Thyroid Association is used (WERNER, 1969)

1. *Primary hypothyroidism*
   - a) Idiopathic myxedema (chronic lymphocytic thyroiditis, atrophic variant)
   - b) Aplasia or ectopism of thyroid tissue
   - c) Destruction of thyroid tissue by
     - Surgery
     - Radioiodine
     - External radiation
   - d) Drug-induced hypothyroidism
     - Thionamide drugs
     - Perchlorate
     - Iodide (high doses)
     - Others (lithium etc.)
   - e) Inborn errors of hormonal synthesis (familial goiter)
   - f) Endemic cretinism, hypothyroid variant
2. *Secondary hypothyroidism*
   - a) Thyrotropin deficiency
     - Isolated
     - Panhypopituitarism
   - b) Thyrotropin-releasing hormone deficiency

effect. In secondary hypothyroidism the thyroid responds well to exogenous TSH. Myxedema describes alterations in the skin that are often but not always found in hypothyroid patients. Some authors use the term interchangeably with hypothyroidism, others only for severe forms of the disease.

Endemic cretinism occupies a special place in the classification. Hypothyroidism does not explain all its features and incidentally is not always present in endemic cretins (p. 215).

Our nomenclature roughly follows that adopted by the American Thyroid Association (WERNER, 1969), but the classification is somewhat different (Table 1).

2. Primary Hypothyroidism

a) Etiology

α) Idiopathic Hypothyroidism (synonyms: spontaneous or primary thyroid atrophy; chronic thyroiditis, atrophic variant)

This is the commonest form of spontaneous hypothyroidism in the adult and is now considered the end result of a chronic inflammatory process. Of the patients with this form of hypothyroidism 83% have serum antibodies to components of thyroid tissue, an incidence only surpassed in chronic lymphocytic thyroiditis (97%). Most authorities now agree that idiopathic hypothyroidism is the end result of a process basically similar to chronic lymphocytic thyroiditis (DONIACH, 1963; BASTÉNIE, 1972) and that it is an autoimmunological disease. Just how many patients with chronic lymphocytic thyroiditis progress into myxedema is not known. Only a few patients with idiopathic hypothyroidism give a history of a transient goiter, such as would be expected if they had suffered from classic chronic lymphocytic thyroiditis. Therefore the suffixes asymptomatic or atrophic variant have been coined to differentiate this disease from the ordinary chronic lymphocytic thyroiditis. Patients with idiopathic hypothyroidism and those with chronic lymphocytic thyroiditis often give a family history of the disease. Relatives of patients have an increased incidence of serum antibodies to thyroid tissue, but only a few have overt thyroid disease.

Women are afflicted with idiopathic hypothyroidism five times as frequently as men. More than 70% of cases are diagnosed after the age of 50 (BASTÉNIE, 1972). Children, however, are also affected in rare cases. A few cases of Graves' disease progressing spontaneously into idiopathic hypothyroidism later have been observed (MEANS, 1963).

BASTÉNIE (1971) has recently described a subclinical or asymptomatic variety of this form of hypothyroidism, which he found associated with early coronary artery disease. Subacute nonsuppurative thyroiditis occasionally causes transient hypothyroidism, but only very few of these patients ultimately develop permanent myxedema (IVY, 1961).

On pathological examination (Fig. 35, p. 225) the thyroid gland of a patient with idiopathic hypothyroidism is usually small in size (< 20 g). The histological picture varies between the extremes of lymphocytic thyroiditis with mostly intact follicles to a dense fibrous tissue with few epithelial cells left (BASTÉNIE, 1972).

β) Hypothyroidism due to Aplasia or Ectopism of Thyroid Tissue (is discussed on p. 166)

γ) Hypothyroidism due to Thyroid Destruction

With the increasing use of radioiodine therapy for thyrotoxicosis this will probably soon be the most frequent cause of hypothyroidism. The original claims by some authors that elaborate calculation of the dose or administration of low doses will prevent this complication of treatment with radioiodine has not been substantiated by careful studies. The prevailing view now is that the dose probably affects the incidence of hypothyroidism after one or two years, but that thereafter an additional 2 to 5% of the patients become hypothyroid each year irrespective of the dose (see section on hyperthyroidism and also the excellent short review by GLENNON, 1972).

The thyroid gland is usually considered quite resistant to external radiation and occasional cases of hypothyroidism after X-ray therapy have been attributed to concomitant autoimmune disease (MARKSON, 1965). More recently GLATSTEIN (1971) has found that of 174 patients with malignant lymphoma who received high-dose radiation to the neck area 44% had elevated TSH levels and 25% became frankly hypothyroid.

Subtotal thyroidectomy for Graves' disease or euthyroid goiter was recognized as a cause of hypothyroidism nearly a century ago by KOCHER (1883). The reported incidence varies widely from study to study (see section on treatment of hyperthyroidism). A high incidence was reported by MICHIE (1972). He found that 49% of 278 patients subjected to surgery for Graves' disease 2 to 6 years previously had become hypothyroid. The incidence of hypothyroidism in his study was clearly related to the size of the thyroid remnant left by the surgeon. This last contention contrasts with

HARGREAVES' (1968) observation that postoperative hypothyroidism correlated more closely with the degree of lymphocytic infiltration of the gland and with serum thyroid antibodies than with the size of the thyroid remnant. MICHIE (1972) found that most of his patients who became hypothyroid did so within 4 months of the operation and that very few later developed myxedema. However, there is no general agreement on this point and others have suggested that the incidence of hypothyroidism probably rises in a cumulative fashion, as after radioiodine therapy (see p. 194).

δ) Drug-Induced Hypothyroidism

The most frequent cause of this form is overdosage of antithyroid drugs during treatment of thyrotoxicosis. Thionamide drugs and perchlorate are both potential causes. Hypothyroidism is occasionally induced by drugs whose primary effect is not on the thyroid gland. Sulfonyl ureas used in the treatment of diabetes have been found to produce hypothyroidism as a side effect; HERSHMANN (1968) has pointed out that these drugs also displace thyroxine from its binding sites on plasma proteins, which lowers the serum thyroxine concentration and may lead to an erroneous diagnosis of hypothyroidism. Since the introduction of lithium into the treatment of manic-depressive illness an increasing number of cases of goiter and/or hypothyroidism due to this drug have been reported (SCHOU, 1968; CANDY, 1972). The thyroid lesion is reversible after discontinuation of lithium. The exact mechanism of action is not yet clear, but so far it has been established that the lithium ion is actively transported into the thyroid cell and inhibits active transports of iodide and colloid droplet formation after TSH stimulation (BERENS, 1970; BURROW, 1971; WILLIAMS, 1971). The basic effect of lithium may be inhibition of thyroidal adenyl cyclase.

In thyrotoxicosis, pharmacological doses of iodine have a rapid antithyroid effect, but the normal thyroid gland usually tolerates large doses of iodine without adverse effects or with only a minimal decrease of hormone secretion (VAGENAKIS, 1973). However, in the past 25 years it has been recognized that occasional euthyroid patients develop severe but reversible hypothyroidism when given pharmacological doses of iodide, e.g. in the treatment of asthma, (PARIS, 1960; BEGG, 1963; MURRAY, 1967). This problem of iodide-induced goiter and hypothyroidism has been exhaustively reviewed by WOLFF (1969). The normal thyroid gland is known to escape from the effects of iodide by shutting off active iodide transport, thereby preventing the build-up of excessive iodide levels in the cell. In susceptible persons this autoregulatory control of active iodide transport fails for unknown reasons. Patients with chronic lymphocytic thyroiditis and patients with Graves' disease who have been treated with radioiodine are particularly prone to the development of iodide myxedema (BRAVERMAN, 1969, 1971). Iodide seems to be particularly inhibitory on thyroid function when it is combined with another drug which has a weak antithyroid effect itself. Many patients with iodide goiter in a study in England were taking a widely prescribed antiasthma preparation (Felsol powder) which contains iodopyrine and phenazone. The latter is a weak antihyroid drug and is probably ineffective by itself, but it greatly potentiates the effect of the iodine component (PASTERNAK, 1969). An endemic form of iodide goiter, usually without hypothyroidism, is known as coast goiter in Japan (SUZUKI, 1965). The anticonvulsant drug aminogluthetimide has occasionally produced hypothyroidism (RALLISON, 1967).

ε) Hypothyroidism due to Inborn Errors of Hormone Biosynthesis

This group of thyroid diseases, also grouped under the name of familial goiter will be discussed in detail in the section on childhood hypothyroidism (p. 167). Deficient production of thyroid hormone means that circulating TSH levels are very high in these patients. The thyroid is therefore under maximal stimulation and goiter develops soon after birth. Since in most other forms of hypothyroidism the gland is small or even nonpalpable, the combination of goiter and hypothyroidism arouses the suspicion of an inborn error of metabolism. In some cases the defect is only partial and hypothyroidism is mild or absent.

b) Incidence of Hypothyroidism

The overall true incidence is difficult to establish. In Zurich the incidence was calculated at 0.25% in outpatients and 0.2% in inpatients. MEANS (1963) cites an incidence of 0.01 to 0.08% for inpatients in the U.S.A., and also states that it is one eighth as frequent as thyrotoxicosis. In centers where radioiodine is the preferred treatment for Graves' disease the incidence of hypothyroidism must be expected to rise in the future. Women are affected 5 to 7 times more frequently than men. The incidence rises with the age of a patient population and may reach 1.9% among geriatric patients.

c) Pathologic Anatomy of Various Organs in Hypothyroidism

In primary hypothyroidism the anterior pituitary is under intense stimulation to secrete TSH. The morphologic counterpart to this is a measurable increase in the size of this gland and an increase in the number of the so-called beta-2 cells, a subclass of basophil cells previously known as amphophil (RUSSFIELD, 1955) or PAS-positive gamma cells (EZRIN, 1959). In long-standing primary hypothyroidism nodular hyperplasia may arise in the anterior pituitary, sometimes developing into true adenoma (MÖSLI, 1968). The gonads remain poorly developed in childhood hypothyroidism. In rare cases, however, premature menstruation and galactorrhea have been observed and ascribed to concomitant increases in the secretion of gonadotrophic hormones and prolactin (see p. 158).

Other morphologic changes in hypothyroidism are found mainly in the skin, the musculature and the skeleton. Strictly speaking, the term myxedema describes the edematous swelling of the corium of the skin with deposits of mucoid material, in particulate acid mucopolysaccharides, and an increase in mast cells. The same changes occur in skeletal and smooth muscles and in the myocardium. Deposits in these structures are not only extracellular but also occur as basophil inclusions in the cells. Skeletal changes occur mainly in childhood hypothyroidism and result in retarded bone age. The epiphyseal growth zones remain noncalcified for a long time, and if ossification does eventually begin, it is irregular, producing the picture of epiphyseal dysgenesis. The abnormal ossification in the hip often leads to impairment of joint function resembling that in Perthes' disease or in osteoarthritis.

d) Clinical Picture in Primary Hypothyroidism

α) History and Mental Changes

The onset is insidious and overt hypothyroidism does not develop until a large part of the thyroid tissue has been destroyed. The pathologic process in the gland precedes the appearance of overt hypothyroidism by years. Hypothyroid patients rarely mention symptoms spontaneously. Since they are often over 60, they are simply considered senile or sometimes even insane by their relatives, and a high index of suspicion is needed to make the diagnosis in mild cases.

One of the earliest complaints is intolerance of cold. The patient feels uncomfortable in a well-heated room, wears more clothes than previously and uses an extra blanket at night. He also perspires less than before.

Changes in mental function are sometimes noticed by the patient himself, often by his relatives. The patients complain of fatigue (not a very specific symptom) and they sleep a lot, preferably in a well-heated room. The mental changes are often quite similar to those in cerebral atherosclerosis. The functions of memory and critical judgement are impaired. In early stages the patient may be capable of good judgment and of making decisions, but his thought processes are slow. The sexual drive is diminished. In later stages a state of mental dumbness with loss of interest in the surroundings and of most previous personality traits prevails. Occasionally the mental changes progress into acute delirium which is clinically indistinguishable from delirium due to exogenous causes, but can be differentiated by the presence of slow waves and low voltage in the EEG. The delirium may progress to stupor and coma. Other patients show changes identical to those in classic manic-depressive or acute schizophrenic psychosis. In some cases this may be due to a true underlying psychosis which becomes manifest in thyroid failure (BLEULER, 1964).

In mild cases mental processes may not be slowed down. Occasionally the symptoms are more similar to those of excitation and even suggest the diagnosis of hyperthyroidism at first.

β) General Appearance

It has been said that hypothyroid patients all look alike. Indeed full-blown cases can be unmistakably recognized at first glance from the typical facies (Fig. 5). The individuality of the

Fig. 5. 72-year-old woman with severe myxedema

facial features is diminished. Mimic movements are few and slow, although still present, in contrast to Parkinson's disease. The patients look sleepy, but happy. No other endocrine disease changes the facies of a person so completely as myxedema.

The voice is characteristically low pitched and hoarse with a nasal undertone, and the speech is slow and monotonous, badly articulated like that of a person under the influence of alcohol. These changes are sometimes so characteristic that the experienced physician can make the diagnosis when speaking to the patient on the telephone.

γ) Skin

Cutaneous vasoconstriction and myxedema makes the skin waxy and pale. Sometimes it has a pink or yellowish undertone due to the deposition of carotene, the hepatic conversion of which to vitamin A is impaired. Carotene can also be the cause of a peculiar malar flush which looks rather like badly applied make-up. Swelling is particularly noticeable in the eyelids and produces narrowing of the palpebral fissures. In addition, the upper eyelid is often slightly ptotic, an effect ascribed to a decreased tone of Mueller's muscle, which is innervated by the sympathetic nervous system. The tongue is thickened and immobile, sometimes even protruding. Scalp hair becomes coarse and fragile, and loses its luster. It falls out easily and frontotemporal baldness ensues. The eyebrows fall out completely or more often only on the lateral part. Myxedematous changes are often found diffusely in the entire skin but sometimes remain localized in a few well-demarcated areas, particularly over the anterior aspect of the legs. Myxedema is characteristically nonpitting, but in the lower extremities true interstitial pitting edema is observed, sometimes to a grotesque degree. The skin is cool, dry, rough, and scaly, with increased cornification on the elbows and knees. Skin changes have recently been quantitatively evaluated by BLACK (1972). He found that the thickness was measurably increased and that the collagen content was normal. The fingernails are thin and brittle and grow slowly. The body temperature falls to 35–36.5°C (95.0–97.7°F), and in extreme cases potentially fatal hypothermia develops. Contrary to popular belief, generalized obesity is only rarely found in hypothyroidism, but localized accumulation of fat over the clavicles does occur (Fig. 13). Beard and pubic hair growth is diminished, although not to the same degree as in hypopituitarism. Wound healing is delayed.

δ) Thyroid Gland

In idiopathic hypothyroidism the thyroid gland is usually not palpable and goiter is only rarely recorded. However, CASSIDY (1970) reviewed 234 cases of nontoxic goiter and made the unexpected finding that 15% were hypothyroid. Thus, the presence of a goiter does not exclude the possibility of hypothyroidism. The association of a large goiter and hypothyroidism is suggestive of an hereditary defect in hormone biosynthesis (p. 167).

ε) Cardiovascular System and Plasma Lipids

In severe myxedema the heart is diffusely enlarged due to myxedematous infiltration of the myocardium, loss of tone, and dilatation of all cavities. Thyroxine treatment alone may bring about a reduction in heart size. In advanced cases, pericardial or pleural effusions and sometimes ascites are present. The heart sounds are diminished. Classically there is bradycardia, but this sign is not always present. Contrary to many textbook statements the blood pressure, in particular the diastolic pressure, is moderately elevated in 50% of cases of hypothyroidism (MEANS, 1963). The stroke volume and cardiac output are diminished, but in contrast to the situation in cardiac failure the arterio-veinous oxygen difference remains normal. Manifest heart failure is infrequent and, when it is present, is often due to additional factors.

In mild cases the electrocardiogram shows flattening, and occasionally inversion, of T-waves. The QT interval remains normal, but the PQ interval and QRS complexes sometimes become wider. In advanced cases the characteristic low voltage appears. This cannot be attributed solely to increased electrical resistance of the myxedematous skin, as measurements with deep-needle electrodes have shown. The electrocardiographic changes are most probably related to the myxedematous changes in the myocardium.

Plasma cholesterol is usually elevated in hypothyroidism, but due to the wide normal range this laboratory sign is of little diagnostic value. The rate of cholesterol synthesis appears to be normal (or even slightly low) in hypothyroidism, and the cause of the high plasma level appears to be slower removal. Triglycerides are also elevated, but frank hyperlipemia is rare. The cause of the hypertriglyceridemia is a low fractional triglyceride removal rate, possibly due to a low postheparin lipolytic activity (NIKKILÄ, 1972; TULLOCH, 1973). KIRKEBY (1972) has recently undertaken a detailed reassessment of serum lipids in hypothyroidism

and compared the findings to carefully matched controls. In addition to the facts already known he has shown that the qualitative distribution of plasma triglycerides and phospholipids in fatty acids is not much changed by hypothyroidism, while in hyperthyroidism a decrease in the proportion of linoleic acid was noted. A diet low in saturated fat may reduce the cholesterol, but not the triglyceride plasma level. Thyroid hormone treatment normalizes both values (O'HARA, 1966).

The relationship of hypothyroidism and the concomitant hypercholesterolemia to coronary artery disease is complex. Autopsy findings of severe myxedema without coronary atherosclerosis have been reported (NICKERSON, 1960 see ref on myxedema coma). Atherosclerosis is rare in juvenile hypothyroidism. Excellent studies by a Belgian group have recently cast come light on the question (VANHAELST, 1967; BASTÉNIE, 1967, 1971). They found that autopsy revealed severe coronary atherosclerosis in 84% of hypothyroid patients, while the incidence in carefully matched controls was only 46%. When patients whose myxedema had been treated were excluded, coronary infarcts were rare in hypothyroidism. Infarcts were recorded in 6 patients, 4 of whom had recently been started on thyroxine medication. Thus it seems that the hypometabolism of hypothyroidism protects these patients from myocardial infarction, despite severe atherosclerosis. Patients with so-called asymptomatic atrophic thyroiditis are at a disadvantage in this respect (BASTÉNIE, 1967). The disease is defined by clinical euthyroidism with normal (or borderline low) thyroid function tests, thyroid serum antibodies and confirmation of atrophic thyroiditis at autopsy. These patients seem to be particularly prone to coronary infarcts, since the syndrome was recorded in 20% of men and 50% of women admitted to hospital for coronary infarction, an incidence much higher than that found in matched controls. Although this statement has since been qualified insofar as asymptomatic thyroiditis was only a risk factor for coronary heart disease in women (BASTÉNIE, 1971), there is still an overall impression that patients with asymptomatic thyroiditis in whom coronary atherosclerosis is not associated with the "protective" effect of hypometabolism have a greater risk of myocardial infarction. Similar conclusions were reached by FOWLER (1967, 1970).

HEINONEN (1972) has expressed disagreement with this concept. In a health survey covering over 3000 people in Finland he identified 53 with severe coronary heart disease. In this group the incidence of thyroid antibodies was no higher than among carefully matched controls. The discrepancy between his results and those of BASTÉNIE will have to be settled by further studies.

One of the clinical consequences is that coronary insufficiency may become manifest when substitution therapy with thyroid hormone is instituted in a myxedematous patient. Treatment should therefore begin with very low doses, and in the patient over 50 years of age it is often necessary to compromise over the final maintenance dose, which should be such that the patient remains borderline hypothyroid (p. 165), because on full substitution many patients suffer from intractable angina pectoris.

ζ) Alimentary Tract

The poor appetite is compensated by the diminished metabolism so that there is no loss in body weight. Difficulty in swallowing and the feeling of a lump in the throat are often early symptoms of hypothyroidism. The submaxillary salivary glands are sometimes moderately enlarged. Obstinate constipation results from the reduced food intake, reduced metabolism and sluggish peristalsis. In 50% of cases there is histamine-refractory achlorhydria. The number of parietal cells in the fundus of the stomach is reduced, and true pernicious anemia is frequently found. The intestinal atony leads to the disturbing symptoms of flatulence and meteorism, and abdominal pains can simulate an ileus. True ileus due to fecal impaction is a much-dreaded complication. The incidence of hiatus hernia is increased.

Morphologic changes in the liver are quite non-specific (KLION, 1971).

η) Lungs

Lung function in hypothyroidism has been studied by WILSON (1960). The main finding was decreased diffusion capacity for CO. More recently, GIORGETTI (1973) has found that the alveolo-arterial difference in partial oxygen pressure during respiration of 100% oxygen is greatly elevated in myxedematous patients. Induction of hyperventilation decreased the alveolo-arterial pO_2 difference and the abnormalities were reversible after thyroid hormone treatment. The most likely explanation for these findings is an increase in intrapulmonary vascular shunts, which may be due to underventilation or atelectasis of small pulmonary segments.

ϑ) Blood

Cutaneous vascoconstriction causes hypothyroid patients to look very pale. This may be why

the early literature described anemia as a frequent complication of hypothyroidism. More recent studies have yielded completely normal hematological findings in two-thirds of patients with hypothyroidism, apart from the fact that most patients have a decreased total red-cell mass associated with the decreased blood volume (TUDHOPE, 1960). Several forms of anemia have to be considered in connection with hypothyroidism.

In so-called "uncomplicated" anemia of hypothyroidism the hemoglobin is moderately low. There is normocytosis or slight macrocytosis (TUDHOPE, 1960). Plasma iron is moderately lowered and the plasma iron turnover rate is decreased. Incorporation of iron into erythrocytes is normal, as is the erythrocyte survival time (KIELI, 1967). The bone marrow may be moderately hypoplastic (AXELROD, 1951). Thyroid hormone substitution therapy reverses all these hematological changes. The pathogenesis of this type of anemia is not entirely clear. Most authors relate it to decreased oxygen requirements and a consequent drop in erythropoeitin levels. Recently it has also been shown that thyroid hormone stimulates hemoglobin biosynthesis in bone-marrow culture (FUHR, 1970). The observation that thyroid hormone stimulates 2,3-diphosphoglyceric acid synthesis in red cells may also be relevant in this context (SNYDER, 1970).

Iron-deficiency anemia with all the associated hematological findings is present in 15% of patients with hypothyroidism. In most cases it can be related to the severe menorrhagia which often accompanies hypothyroidism in women. These patients need iron therapy and hormonal substitution to completely normalize the blood findings.

Pernicious anemia is found in 8% of patients with hypothyroidism, in comparison to 0.35% of matched controls. The incidence is higher in idiopathic hypothyroidism than in hypothyroidism following thyroidectomy or radioiodine treatment (10% as against 4%). The original claims that thyroid hormone substitution improves absorption of vitamin B_{12} (LEITHOLD, 1958) were not confirmed by subsequent studies (TUDHOPE, 1960, 1962). The present view is that these patients suffer from true pernicious anemia and that idiopathic hypothyroidism is pathogenetically related to this disease. The fact that true pernicious anemia is also more frequent in patients with Graves' disease may explain the above association between postsurgical or post-radioiodine hypothyroidism and pernicious anemia. Antibodies to mucosa of the gastric fundus and intrinsic factor are prevalent in patients with idiopathic hypothyroidism, as they are in pernicious anemia. They are rare in control subjects. Patients with pernicious anemia do quite often have serum antibodies to thyroid tissue, however. Gastric mucosal biopsies in hypothyroid patients revealed a reduction in the number of parietal cells and lymphocytic infiltrates (IRVINE, 1962). Nearly 50% of patients with hypothyroidism suffer from histamine-refractory achlorhydria. Both pernicious anemia and idiopathic hypothyroidism are currently viewed as disorders of autoimmunity, possibly related to a generalized defect of immune tolerance. The two conditions are clinically similar and clinical differential diagnosis may be difficult at times. It is advisable to assess the thyroid status of every patient suffering from pernicious anemia. In addition, whenever thyroid hormone and iron substitution do not cure anemia of hypothyroidism, intestinal vitamin B_{12} absorption or serum vitamin B_{12} levels should be measured.

Leukocytes and platelets are normal in hypothyroidism. The erythrocyte sedimentation rate is usually accelerated.

ι) Genital Organs and Gonads

Hypothyroid women often suffer from menorrhagia, but ammenorrhea is also encountered. Sterility in both sexes is common, but occasional pregnancies do occur in hypothyroid women (see STANBURY, 1972, for review). The sexual drive is usually reduced and impotence is not uncommon. A total of 12 cases of amenorrhea and galactorrhea associated with hypothyroidism have been reported (VAN WYK, 1960). They all developed post partum. EDWARDS (1971) has added a further case which occurred when the patient took oral contraceptives. Prolactin levels were high and returned to normal under thyroxine treatment, as did the other clinical symptoms.

Occasionally men suffer from coincident hypogonadism and hypothyroidism (BALZE, 1962).

κ) Muscles and Joints

The strength of the hypotonic and very often pseudohypertrophic musculature is reduced. Muscle pain and stiffness are common complaints. Tapping the muscle produces the typical pseudomyotonic reaction in the form of a slow contraction changing into a hard muscular ridge. These contractions never outlast the voluntary innervation as they do in myotony.

The tendon reflexes are characteristically very sluggish, and the recording of the ankle

jerk shows a typical curve, which is useful in diagnosis. Hypothyroidism can produce severe histological changes in muscle characterized by peculiar mucoid deposits between and within muscle cells. The serum creatine phosphokinase (CPK) is elevated, indicating muscular damage (GRAIG, 1965). Similar changes occur in smooth muscle. They may lead to degenerative changes in the media of large blood vessels and to rupture of the aorta. Hypothyroidism may be associated with myasthenia gravis (BRONSKY, 1967; Chap. XVII).

Arthralgia, joint swelling, synovial thickening and joint effusions are sometimes the prominent features of hypothyroidism, and such patients are referred to rheumatology units before the diagnosis of hypothyroidism is established (BLAND, 1970). Most of the symptoms disappear during thyroid hormone substitution therapy.

λ) Nervous System (see also p. 155)

Cerebellar dysfunction with ataxia and intention tremor occur occasionally. Sensory disturbances are more common, and the patients often complain of numbness and paresthesias. Compression of the median nerve with carpal tunnel syndrome is often encountered. The protein concentration in the cerebrospinal fluid, particularly that of gamma globulin, is frequently elevated.

Partial deafness is a frequent finding in adult hypothyroidism. The pathogenesis is not quite clear. It appears to be due to a conductive defect associated with swelling in the eustachian tube. Sensorineural deafness has been ascribed to degenerative changes in the mucosa of the cochlea (RITTER, 1967) and sometimes to changes in the labyrinthine fluid composition.

μ) Kidney, Electrolytes and Body Fluids

Morphologically the kidneys may show deposits of mucopolysaccharides in the intercapillary space and in the basal membranes of the tubuli. Electron microscopic examination reveals thickening of the glomerular and tubular basal membranes (CASSANO, 1964). SALOMON (1967) has performed repeated biopsies and shown that these changes are reversible under appropriate treatment with thyroid hormone. Clinically, oliguria is sometimes observed and is attributed to a low fluid intake. Slight proteinuria is often present. Laboratory examination of kidney function has revealed a decrease of glomerular filtration rate and renal plasma flow.

These changes in kidney function rarely lead to overt electrolyte disturbances. Occasionally, however, particularly in severe myxedema and in myxedema coma, dangerous hyponatremia develops (AIKAWA, 1956). Superficially this form of hyponatremia resembles that seen in the syndrome of inappropriate secretion of antidiuretic hormone (ADH), since plasma osmolality is low and urine osmolality high. The disturbance was therefore initially ascribed to inappropriately high levels of antidiuretic hormone (IVY, 1965; PETTINGER, 1965). Further studies however have shown that the pathogenesis of this electrolyte disturbance is far more complex and cannot yet be fully explained. So far it is established that the hyponatremia is dilutional, i.e. total exchangeable body sodium is normal or slightly increased and total body water is definitely increased. Although resetting of the osmoreceptors to a lower level may play some role, and maintain the serum sodium at borderline low levels by the secretion of ADH, the control of ADH secretion is not lost. When given a water load these patients are perfectly capable of diluting their urine to 60 mOsm/l (DISCALA, 1971; DERUBERTIS, 1971), although the peak urine flow and the free water clearance are lower than in controls. The minimum urine osmolality reached is slightly higher than in normal persons. The best explanation for the hyponatremia of hypothyroidism so for available is that it is due to a decreased volume and solute delivery to the distal diluting tubular segment (DERUBERTIS, 1971; DISCALA, 1971). Decreased glomerular filtration and increased proximal sodium reabsorption may both contribute to this decreased volume delivery to the distal tubule.

It must be added that a relative lack of corticosteroids may also be involved in the hyponatremia, but this is less well established (see DISCALA, 1971, for a short review).

ν) Diagnostic Weight of the Various Clinical Findings

BILLEWICZ (1969) has made an extensive mathematical analysis of the significance of the various clinical findings for the diagnosis of hypothyroidism. He has developed a very useful diagnostic index allowing confirmation or rejection of the diagnosis of hypothyroidism with great accuracy on clinical findings alone, which may thus render expensive laboratory tests unnecessary. Symptoms which are significant for the distinction between hypo- and euthyroidism are diminished perspiration, preference for warm ambient temperatures, hoarseness of the voice, paresthesias, and dry skin. The most significant signs are slowness of the ankle

jerk, slow movements on undressing, and dry rough skin on the forearms. Contrary to popular belief, weight gain has practically no diagnostic value.

ξ) Thyroid Function Tests

Serum thyroxine can vary from the low normal range to pratically unmeasurable levels, depending on the severity of the disease. In primary myxedema the serum triiodothyronine is relatively less lowered than the serum thyroxine, because the high TSH level favors the synthesis of triiodothyronine over that of thyroxine (McConnon, 1971). The resin T_3-uptake test yields results in the hypothyroid range. The ankle reflex time is prolonged (p. 241) and the basal metabolic rate is below -15%. Radioiodine uptake is low at two hours and not much higher at 24 hours. Administration of TSH (p. 242) does not increase the serum thyroxine or radioiodine uptake. TSH levels are invariably very high in primary hypothyroidism. Indeed, the serum TSH seems to be the most sensitive index of thyroid failure, since it is often elevated in borderline cases where the other tests still yield values in the normal range (p. 161). Positive tests for serum antibodies to thyroid antigens provide strong support for a diagnosis of primary hypothyroidism (Vallotton, 1967). Several authors have attempted to assess the reliability of the various thyroid tests in establishing the diagnosis of hypothyroidism (Rives, 1965; Fitzgerald, 1966; Gordon, 1970; Chew, 1962). The serum hormone level (serum thyroxine or protein-bound iodine), the basal metabolic rate and the 24-hour radioiodine uptake all had an almost equally high rating in their agreement with the final diagnosis (based on all tests and on the clinical findings). The resin T_3-uptake, the ankle reflex time, and the serum cholesterol were less useful in diagnosis, but it must be added that in a recent study the ankle reflex time had the highest rating (Fraigu, 1972).

Intense stimulation of the remaining thyroid tissue by TSH leads to a relative increase in triiodothyronine secretion compared with that of thyroxine. It has even been claimed that primary hypothyroidism can be distinguished from secondary failure by the relatively normal levels of serum triiodothyronine in primary hypothyroidism (McConnon, 1971).

3. Secondary Hypothyroidism

Secondary hypothyroidism occurs most frequently in the setting of panhypopituitarism, the causes of which are listed in Chap. V (p. 92f.). Occasionally, TSH deficiency is the predominant feature of a pituitary lesion, and gonadal and adrenal function are less compromised (Sampson, 1954; Sawin, 1966; Odell, 1966). In such cases it may be difficult to differentiate secondary hypothyroidism from primary myxedema on clinical grounds.

Miyai (1971) has described a rare cause of secondary hypothyroidism in two hypothyroid children of a consanguineous marriage. They both suffered from congenital isolated deficiency of TSH secretion with intact secretion of the other pituitary hormones.

It seems likely that in the future some cases of secondary hypothyroidism will have to be classified as due to hypothalamic rather than to pituitary damage. Such cases have recently been well documented by Pittman (1971) and Shenkman (1972). The patients were able to secrete TSH following pituitary stimulation effected by the injection of thyrotropin releasing hormone (TRH), and the defect was thought to be at the level of TRH secretion. The availability of synthetic TRH and of a TSH radioimmunoassay will probably make the TRH stimulation test (p. 242) a routine procedure in the assessment of panhypopituitarism, particularly in childhood. Most children with idiopathic deficiency of growth hormone and TSH tested so far have shown a brisk rise in serum TSH after injection of TRH. They presumably suffer from hypothalamic damage (Costom, 1971; Foley, 1972, 1972b).

The differentiation of secondary from primary hypothyroidism is of more than academic interest and influences the choice of treatment for the patient. In secondary hypothyroidism, due to the frequent coexistence of adrenocortical insufficiency, thyroid hormone substitution may provoke a potentially fatal adrenal crisis if corticosteroids are not given simultaneously.

The clinical sign most useful in differentiation is the white, almost transparent, and finely wrinkled skin of secondary hypothyroidism, as opposed to the coarse, thick, dry, and slightly yellowish skin of primary myxedema. Amenorrhea, atrophy of the testes, adrenal insufficiency and tendency to hypoglycemia all are points indicative of secondary thyroid failure. Pubic and axillary hair are sparse in primary hypothyroidism, but their complete absence is additional support for the diagnosis of secondary hypothyroidism. Urinary corticosteroid excretion is moderately diminished in primary myxedema, but very low in panhypopituitarism. In hypopituitarism the thyroid gland retains a certain degree of autonomic function, so that thyroid failure is

rarely as severe as it sometimes is in primary myxedema. In complete failure of the thyroid gland the basal metabolic rate may fall to −40%, whereas in secondary failure it is rarely below −30%. The presence of serum antibodies to thyroid antigens provides strong support for the diagnosis of primary hypothyroidism (VALLOTTON, 1967). On average, serum cholesterol is less elevated in secondary than in primary hypothyroidism, but in the individual patient this sign is of little value for differentiation.

The diagnosis of secondary hypothyroidism is established by the finding of a low or low normal serum TSH concentration. In primary hypothyroidism serum TSH is always elevated. Where TSH measurements are not available, the TSH stimulation test will show a good response of the serum thyroxine and the radioiodine uptake to repeated injections of bovine TSH (p. 242). It must be added that long-standing primary hypothyroidism may impair the function of the anterior pituitary (BRAUNMAN, 1968; LESSOF, 1969) and the term myxedema of the pituitary is sometimes used to describe this condition.

4. Borderline Hypothyroid States

The severity of hypothyroidism can vary between barely impaired thyroid function and complete failure of hormone secretion. The diagnosis of slight hypothyroidism is usually very difficult and should never be made on the basis of a single abnormal test. The clinical picture and several thyroid function tests should be evaluated. It should be borne in mind that each of these tests has a relatively wide "normal" range, and that therefore a value in the low normal range may mean real hypothyroidism for one patient but euthyroidism for another. The most sensitive index of mild primary hypothyroidism currently known is probably the serum TSH concentration, which is often elevated when most other tests still yield results in the normal range. If the TSH radioimmunoassay is not available, the TSH stimulation test (p. 242) is sometimes very helpful.

a) Hypometabolism without Hypothyroidism

This condition is found in patients with anorexia nervosa and during starvation. Serum thyroxine and radioiodine uptake are usually normal, indicating that the lowered basal metabolic rate is not due to thyroid deficiency. The existence of a "functional" type of hypopituitarism as an adaptive phenomenon during starvation remains to be established. A slight decrease in the basal metabolic rate may occur in adrenocortical insufficiency and in male hypogonadism.

b) Borderline Secondary Hypothyroidism, Lack of TSH Reserve

A syndrome with some features of hypothyroidism, such as cold intolerance, fatigue, constipation, anemia and menstrual irregularities, was described in the nineteenth century and termed "myxoedeme fruste" or "hypothyroïdie bénigne chronique" (HERTHOGE, 1899). More recently this condition has been ascribed to resistance of the peripheral tissues to thyroxine (KURLAND, 1955; GOLDBERG, 1960), but subsequent studies have not confirmed this concept (LEVIN, 1960; SIKKEMA, 1960; JEFFERIES, 1961). WYSS (1963) and STUDER (1964) have observed that patients with these symptoms often show a diminished rebound of radioiodine uptake after a 7-day course of carbimazole und have suggested that the condition is due to a diminished TSH reserve. This TSH reserve test has not, however, been reproduced in other laboratories and has been abondoned (POWELL, 1966; SCHNEEBERG, 1966). The syndrome therefore needs to be reevaluated by measuring serum TSH levels following TRH stimulation.

c) Diminished Thyroid Reserve (JEFFERIES, 1956)

This formerly rather vague syndrome can now be more exactly defined as the presence of elevated TSH levels together with normal thyroid function tests and only intermittent slight clinical symptoms of hypothyroidism. It is most often observed after radioiodine treatment for hyperthyroidism (SLINGERLAND, 1972; STERLING, 1971), in chronic lymphocytic thyroiditis (GHARIB, 1972) or after subtotal thyroidectomy (HEDLEY, 1972). A borderline euthyroid state is often maintained in these circumstances despite a low serum thyroxine level, thanks to a normal or slightly elevated serum triiodothyronine concentration (STERLING, 1971). In an analogous situation in endemic goiter DELANGE (1972) has measured triiodothyronine concentrations up to 250% of normal.

5. Myxedema Coma (see Table 2)

Myxedema coma was recognized as the end stage of hypothyroidism over 90 years ago (ORD, 1880), but only in the last decades has it received appropriate clinical attention. An exhaustive review of 122 cases has been prepared by LEON-SOTOMAYOR (1964).

Table 2. Treatment of myxedematous coma

1. If arterial pCO_2 is over 50 mm Hg, endotracheal intubation or tracheostomy and assisted respiration. Relief of bronchial airway obstruction.
2. Administration of corticosteroids in an intravenous drip, e.g. cortisol hemisuccinate 100 mg in the first 3 hours, then 10 mg per hour until the patient regains consciousness.
3. Triiodothyronine 12.5 µg every 12 hours by i.v. route, or if not available, by nasogastric tube.
4. If hypoglycemia is present, correct with 50 ml 40% glucose in water. Maintain blood sugar by an infusion of glucose made isotonic or hypertonic in saline. Glucagon may also be given in a dose of 1 to 2 mg every hour.
5. If hyponatremia is present, treat with either hypertonic NaCl infusions or with fluid restriction or both.
6. In case of severe arterial hypotension give a slow infusion of plasma or albumin in physiologic NaCl. Norepinephrine or isoproterenol may be tried, but involve the risk of cardiac arrythmias.
7. Careful slow rewarming by not more than 1 °C (1.8 °F) per hour, without heating blankets.
8. If available, institute continuous ECG monitoring. Cardiac glycosides can be given if overt heart failure is present, but little is known about the proper dose in myxedematous coma.
9. If infection is established give an antibiotic.

The lack of thyroid function is compatible with most vital functions, although at a diminished level. Precipitating factors, such as exposure to cold, infections, treatment with barbiturates or phenothiazines, are therefore necessary to lower body temperature, impair alveolar ventilation and to send the patient into coma. Severely hypothyroid patients fall into progressively longer periods of deep sleep until they can no longer be aroused. The body temperature falls to 32–35 °C (89.6–95 °F). It must be recalled here that hypothermia can only be recorded if a special thermometer is used. Severe electrolyte and body fluid disturbances are always present, particularly hyponatremia (see p. 159 for the pathogenesis of hyponatremia). Pericardial and pleural effusions are often found. Bradycardia and arterial hypotension are usual. The tendon reflexes show extremely slow relaxation.

An important factor contributing to the coma is alveolar hypoventilation leading to elevated arterial carbon dioxide tension. The pathogenesis is not entirely clear, but impairment of the medullar respiratory center function, disturbances of neuromuscular transmission, weakness of respiratory muscles, elevation of the diaphragm due to abdominal gas, and accumulation of bronchial mucus may all be contributory factors. The carbon dioxide retention usually precedes hypothermia. The latter may cause disturbances of atrioventricular conductance and severe arrhythmia.

Patients in myxedema coma should always be considered as being in a state of adrenocortical insufficiency with the corresponding lack of resistence to stress of any kind. In primary hypothyroidism hypoglycemia is usually not present, but there may be severe hypoglycemia when myxedema coma is superimposed on panhypopituitarism.

Primary and secondary myxedema coma are difficult to differentiate on clinical grounds. Thin, finely wrinkled, and parchment-like skin, absence of pubic and axillary hair and atrophic testes point to secondary hypothyroidism, while thick skin, edema of the legs and face and a large tongue are indicative of primary hypothyroidism. Distinction between the two forms is irrelevant to the immediate treatment, and the exact nature of the endocrine defect can be established at leisure after the patient has recovered.

Besides the measurement of body temperature with a special termometer the following laboratory tests should be ordered in a case of myxedema coma: Hemoglobin or hematocrit and red cells, blood sugar, serum electrolytes and urea, arterial oxygen and carbon dioxide tension and oxygen saturation of the hemoglobin. A blood culture, a chest X-ray and a urine culture should be obtained to rule out infection which may be present despite hypothermia and low white cell counts. Blood should also be taken for thyroid function tests (serum thyroxine, resin T_3-uptake test), but therapy should not be delayed if their results are not immediately available. Whenever possible patients with myxedema coma should be admitted to an intensive care unit and treatment started immediately. Thyroid hormone substitution is started with triiothyronine, which has a more rapid onset of action than thyroxine. The hormone can be given by gastric tube, but since intestinal absorption may be impaired due to shock, intravenous administration is preferable. There are no commercial preparations available for intravenous use and the solution has to be prepared by the hospital pharmacy as follows: the sodium salt of triiodothyronine is weighed and dissolved in a few drops of 0.1 N NaOH. The solution is then diluted in 5 ml of 1 or 2% solution of human serum albumin in 0.15 M NaCl and sterilized by filtration through millipore. Alternatively, sterile instruments and dilution solution can be used. For the first two or three days 12.5 µg up to a maximum of 25 µg triiodothyronine are given by injection every 12 hours. Overdosage of thyroid hormone carries the risk of fatal cardiac arrhythmia and coronary infarction, but in desperate cases of coma several

authors have recommended higher doses of up to 100 μg triiodothyronine every 12 hours. HOLVEY (1964) has successfully treated 7 patients with single doses of 100 to 500 μg L-thyroxine by the i.v. route followed by lower maintenance doses. Recent studies in noncomatous hypothyroid patients have confirmed that large intravenous thyroxine doses (up to 750 μg initially) are well tolerated and lead to a much more rapid restoration of the deranged metabolic functions (RIDGWAY, 1972). It remains to be seen whether this treatment can be safely recommended for the comatose patient.

Although it is difficult to establish the presence or absence of adrenocortical insufficiency in myxedema coma, corticosteroids should always be administered in generous doses for the first few days of treatment. The recommended dosage is 100 mg of a water-soluble cortisol preparation (e.g. cortisol hemisuccinate) in slow intravenous infusion over the first three hours, followed by 10 mg every hour until the patient regains consciousness. A water-soluble prednisolone preparation in a dose four times lower may be substituted for the cortisol preparation.

Hypoglycemia, if present, should be treated by intravenous infusion of glucose. This may present a problem if the patient has concomitant hyponatremia, since the usual 5% glucose solution will add a large quantity of free water and may aggravate water intoxication. Glucose should therefore be given in a concentrated solution in such cases, or in a glucose solution made 0.15 or even 0.3 M in NaCl by the addition of NaCl.

Hyponatremia may necessitate either fluid restriction, as in the case of true inappropriate secretion of ADH, or administration of hypertonic NaCl solution. Since the total body sodium is not known in an individual case, it is impossible to know which measure to apply first, and it may be necessary to proceed by trial and error.

Arterial hypotension is another difficult therapeutic problem. Therapy can begin with careful slow infusion of plasma or albumin solutions. Pressor amines such as norepinephrine have been recommended, but two facts limit their usefulness. The peripheral vasculature is already maximally constricted in myxedema coma, and pressor amines will have little additional effect. Moreover, they can induce cardiac arrhythmias. In theory, beta-receptor stimulants such as isoproterenol ought to be more useful, but their use in myxedema coma has not yet been assessed to our knowledge.

Hypothermia is treated by slow rewarming at a rate of not more than 1 degree C (1.8 degree F) per hour, avoiding the use of a heating blanket. If rewarming is too rapid, sudden peripheral vasodilatation may produce irreversible shock.

Respiratory acidosis with an arterial pCO_2 of over 50 mm Hg necessitates an endotracheal or a tracheostomy tube and appropriate respiratory assistance.

The prognosis of myxedema coma is very grave. The condition has a fatal outcome in 40% of cases despite all attempts at treatment.

6. Clinical Differential Diagnosis of Hypothyroidism

The diagnosis of severe hypothyroidism is simple in most cases, but a borderline case may escape the attention of even the experienced doctor. Several studies have estimated that an average of 3 to 4 years elapses between the onset of the first symptoms and the diagnosis of hypothyroidism.

Patients with acute glomerulonephritis or with the nephrotic syndrome may occasionally be mistakenly thought to be suffering from hypothyroidism. Pallor, edema, a puffy face, cardiomegaly and anemia are features common to both hypothyroidism and chronic renal disease. Albuminuria may be present in hypothyroidism, but not hematuria. The fact that low serum-binding proteins in the nephrotic syndrome lead to a decrease of the serum hormone level makes the differentiation even more difficult. In terminal renal disease the renal iodide clearance is greatly reduced, but despite this, thyroidal radioiodine uptake is usually normal, probably due to a concomitant increase in the serum iodide level (p. 152). FANKHAUSER (1968 see p. 254) has described mild secondary hypothyroidism of obscure origin in one third of patients with severe chronic renal insufficiency.

Encephalitis with stupor has occasionally been mistaken for hypothyroidism. A slight increase in the protein concentration of the cerebrospinal fluid, as is found in hypothyroidism, may help to confuse the picture, but in general fever and tachycardia are strong arguments against the presence of hypothyroidism. True secondary hypothyroidism due to hypothalamic damage may occur in encephalitis. Parkinson's disease may sometimes mimic hypothyroidism. As outlined in a previous section (p. 157) true pernicous anemia is often found in patients with primary hypothyroidism. Pallor, atrophic muscosa of the tongue, and achlorhydria and paresthesias are frequent in both conditions. In myxedema the tongue is large, whereas it is of normal size in pernicious anemia.

7. Course and Prognosis of Hypothyroidism

Myxedema takes an extremely chronic course. Complete lack of thyroid tissue is compatible with life for some years. After a long period of chronic illness hypothyroidism ends in death in myxedema coma, which is often precipitated by infections, exposure to cold, or administration of sedatives (p. 161). Cases who remain untreated for years are rarely encountered today. The famous report of the Myxoedema Commission (1888) stated that the life expectancy of a patient with untreated myxedema was 10 to 15 years after onset of the disease. Adequate treatment often restores all functions to normal and some famous cases have survived for many years. One of RAVEN's patients (1894, 1924) had been ill and bedridden for 20 years. She recovered promptly after treatment and lived another 30 years, to die at the age of 94. BURGESS (1946) treated a patient successfully over 52 years.

According to MEANS (1963), full-blown myxedema is always due to complete athyroidism. Even small remnants of thyroid tissue may maintain a borderline thyroid function, until they too become atrophic. Since destruction of the thyroid tissue is a slow process in primary idiopathic hypothyroidism, the severity of myxedema is always correlated to the duration of hypothyroidism. Acute spontaneous hypothyroidism has frequently been reported, but it has not been established whether this occurs in primary idiopathic hypothyroidism. Spontaneous remissions are seen after thyroiditis and sometimes after subtotal thyroidectomy. After thyroidectomy and after radioiodine treatment the signs of hypothyroidism may develop quite rapidly.

8. Treatment of Hypothyroidism (Table 3)

Na-L-thyroxine is our drug of choice for long-term substitution therapy. It is relatively cheap and has a constant and well-standardized potency. Its absorption after oral administration is about 70% in both euthyroid and hypothyroid subjects (HAYS, 1968; READ, 1970). EVERED's (1973) careful studies in which the dose of thyroxine was slowly increased until the elevated TSH levels of primary myxedema had returned to normal (probably the best criterion for euthyroidism) have shown that the full replacement dose is 100–200 µg daily. The doses of 200–400 µg recommended in most textbooks are too high and produce slight hyperthyroidism. Oral thyroxine takes effect after about 3 to 5 days (RIDGWAY, 1972) and the effect slowly disappears 7 to 10 days after cessation of treatment. The serum half-life is about 7 days. This slow disappearance is an advantage in most cases. It allows administration of the drug in a single daily dose. In cases where the patients are unreliable, the drug can be given under the surveillance of a doctor or nurse every 7 days in a dose of 1 to 2.5 mg (BERNSTEIN, 1969).

Since the availability of L-thyroxine the desiccated thyroid preparations have lost much of their popularity, but are still often used in the U.S.A. Although it must be conceded that some brands are very reliable in their hormone content and have been used in the same dose over years, the fact that most pharmacopeias require only a statement of their iodine content has limited their usefulness. Since desiccated thyroid contains variable amounts of mono- and diiodotyrosine and iodide as well as thyroxine and triiodothyronine, a constant iodine content does not guarantee a constant hormone content. Some preparations have shown great variability in intestinal absorption, and others have lost their potency during storage.

Triiodothyronine, with its serum half-life of 1 day, has a more rapid onset of action within a few hours to 3 days. After discontinuation of treatment its effect ceases within 3 to 5 days. These properties are useful in the treatment of myxedema coma, in the triiodothyronine suppression test (p. 242) and in cases where it is necessary to interrupt and resume therapy quickly for radioiodine uptake tests or for radioiodine treatment of thyroid cancer.

Recently, thyroid hormone preparations containing a mixture of the two hormones thyroxine and triiodothyronine in a ratio of 4:1 have become commercially available. The claim that they allow more "physiological" and better tolerated treatment has been disproved by a carefully controlled study (SMITH,

Table 3. Preparations for thyroid hormone replacement therapy and their dosage

	Mean adult full replacement dose (day)	Half-life in plasma	Onset of effect (days after start of treatment)	Duration of effect after cessation of therapy
Na-L-thyroxine	0.1 –0.2 mg	7 days	4 days	10 days
Na-L-triiodothyronine	0.06–0.12 mg	1 day	¼ to 3 days	5 days
Desiccated thyroid	60–180 mg	–	4 days	10 days

1970). Surprisingly, this mixture produces unphysiologically high triiodothyronine levels in serum, while substitution with thyroxine alone produces near-normal triiodothyronine levels (SURKS, 1972; EVERED, 1973).

The full substitution dose for thyroxine is 100–200 μg in most adult patients, but the range is 100–600 μg. A dose of 60 μg of triiodothyronine is about equipotent to one of 100 μg of thyroxine. In mild or moderate cases the initial dose is 50 μg of thyroxine per day. After one or two weeks it is increased by increments of 25 μg until optimal substitution is reached. In severe myxedema, in patients over 60 years of age, and in cases of coronary heart disease the initial dose should be low, e.g. 12.5 μg or at most 25 μg per day. The dose is increased by small increments at intervals of not less than 2 weeks until satisfactory substitution is achieved. In older patients, especially in the case of coronary atherosclerosis, it is customary not to aim at full substitution, but to keep the patients borderline hypothyroid. If angina pectoris, palpitations or cardiac arrhythmias arise at the onset of therapy, treatment is stopped and resumed at half the dose after a few days.

The optimum substitution dose should be determined mainly by the clinical state of the patient. Cold intolerance and fatigue should disappear and the pulse should become normal. Palpitations, tachycardia, loss of weight, nervousness and insomnia are signs of overdosage. Laboratory tests may sometimes be necessary to determine the proper dose. The ankle reflex time has proved to be a useful and quick indicator of therapeutic effectiveness. Other authors prefer to measure the basal metabolic rate. Most textbooks recommend that the serumthyroxine should be in the borderline hyperthyroid range during optimal substitution therapy with thyroxine. Recent studies, however, recommend to maintain the serum thyroxine in the normal range (EVERED, 1973). Serum thyroxine is very low (1–2 μg/100 ml) when the patient is given triiodothyronine and should be in the normal range when desiccated thyroid is given.

When the optimal dose has been determined for a patient it should be given for the rest of his life, and patients should be carefully instructed not to discontinue treatment. Physical activity and cold weather cause a moderate increase in the hormone requirement and the dose should be varied accordingly. Early in the treatment patients often tend to forget to take their medication regularly or discontinue treatment completely. They should therefore be seen at frequent intervals at first. Later, six-monthly check-ups are sufficient, but the doctor has a responsibility to follow up the patients throughout their lives.

Concomitant heart failure should be treated with cardiac glycosides. If the patients are not fully substituted with thyroid hormone, the digitalis dose should be kept low.

Secondary hypothyroidism needs the same treatment as primary hypothyroidism with the important exception that substitution with cortisone is mandatory and should be started before thyroxine therapy in order to avoid an adrenal crisis. The treatment of myxedema coma is discussed on p. 161.

E. Hypothyroidism in Childhood

A. PRADER, A. LABHART, and H. BÜRGI

Hypothyroidism in childhood deserves a special section because of its high incidence and particular features, notably its effects on growth and mental development. After diabetes mellitus hypothyroidism is the most common endocrine disorder of childhood. It is more frequent than hyperthyroidism in children,

Table 4. Causes of hypothyroidism in childhood

I. Primary hypothyroidism

1. Thyroid aplasia, ectopic and hypoplastic thyroid (congenital, nonfamilial thyroid aplasia, sporadic cretinism).
2. Acquired childhood and neonatal hypothyroidism.
   a) Treatment of mother during pregnancy with antithyroid drugs or excess iodide (reversible);
   b) Idiopathic hypothyroidism (spontaneous primary thyroid atrophy; chronic thyroiditis, atrophic variant).
3. Genetic defects in thyroid hormone biosynthesis and thyroid hormone end organ response (familial goiter with hypothyroidism).
   a) Failure of active iodide transport;
   b) Defect of organic iodination. When associated with nerve deafness: Pendred syndrome;
   c) Defect in iodotyrosine coupling;
   d) Deficiency of iodotyrosine dehalogenase;
   e) Deficiency of thyroidal protease;
   f) Abnormal plasma iodopeptides;
   g) Unresponsiveness of thyroid to TSH;
   h) Unresponsiveness of peripheral tissues to thyroid hormone.
4. Endemic cretinism, hypothyroid variant.

II. Secondary hypothyroidism

Any of the defects below can be due to a lesion either of the anterior pituitary or of the hypothalamus.

1. Tumors of the hypothalamus or the pituitary gland;
2. Inflammatory, granulomatous or traumatic lesions of the hypothalamus or pituitary gland;
3. Idiopathic deficiency of TSH secretion, often combined with defective growth hormone secretion. Probably hypothalamic in origin in most cases;
4. Isolated hereditary TSH deficiency (rare).

while in the adult the reverse is true. The clinical features peculiar to childhood hypothyroidism arise from the fact that in addition to the effects known in the adult it severely impairs physical and mental development. The resulting picture is so striking that it is usually easy to decide in an adult hypothyroid patient whether his hypothyroidism started in childhood or later.

A classification of childhood hypothyroidism is given in Table 4. The individual causes of childhood hypothyroidism are discussed below.

1. Aplastic, Hypoplastic and Ectopic Thyroid Gland

This is the most common cause of hypothyroidism in children. Thyroid ectopism has been recognized more and more frequently since all "athyrotic" children have been carefully scintiscanned for iodine-concentrating ectopic thyroid tissue. About 50% of these children are found to have a retrolingual thyroid (Fig. 6). The thyroid gland arises during embryogenesis from the thyroglossal duct, a ventral outgrowth of the cranial entoderm. Ectopic thyroid tissue may be found anywhere along the path of this anlage down to the final location of the gland, sometimes even far below the normal site of the gland, e.g. in the lower mediastinum (Fig. 7). The most common location is the base of the tongue as a so-called retrolingual goiter, which is usually very small, but is occasionally large enough to produce dysphagia and other local symptoms (Fig. 6). Only 17% of patients with a retrolingual thyroid demonstrated by scintigraphy are hypothyroid, but if the ectopic thyroid is the only thyroid tissue, hypothyroidism is relatively common. In the early years of life intense stimulation of ectopic thyroid tissue may suffice to maintain marginal euthyroidism,

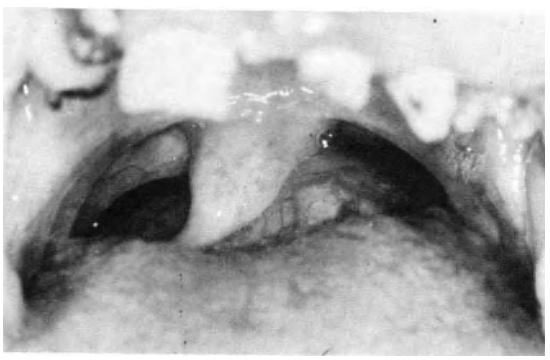


Fig. 6. Lingual goiter in a 42-year-old woman with congenital hypothyroidism. (From M. P. KÖNIG: Die kongenitale Hypothyreose und der endemische Kretinismus, Springer 1968)

but in many cases hormone supplies fail to meet the demands in the growing organism, e.g. in puberty, and hypothyroidism follows.

The radioiodine turnover in the stimulated ectopic retrolingual tissue may be greatly accelerated. Thus radioiodine uptake is rapid, but the tracer leaves the gland again at a fast rate. Scintiscanning should therefore be performed in an early phase of the iodine uptake. Due to the rapid turnover the level of radioactive protein-bound iodine (PB ^{131}I) is fairly high after 24 hours. The salivary glands, which also concentrate iodide, have to be carefully shielded during scintiscanning to avoid false-positive results.

Surgery is not normally necessary in retrolingual goiter. Treatment with thyroxine suppresses the elevated TSH levels, and the hyperplastic thyroid tissue is deprived of its growth stimulus and shrinks. Only if this therapy fails is surgery indicated (NEINAS, 1973; see p. 283).

Unilateral aplasia of a thyroid lobe is rare. It is occasionally simulated by a toxic nodule suppressing the activity of the remaining tissue. Scintiscanning after TSH injection reveals the dormant tissue on the contralateral side in such cases.

The thyroglossal duct sometimes gives rise to cysts, which occasionally become infected. When they are treated by surgery the whole of the thyroglossal duct should be removed to avoid recurrences. The possible sites of ectopic thyroid tissue are shown in Fig. 7.

Thyroid tissue in an ovary, so-called struma ovarii, is part of a teratoma. It is very rare and is seldom diagnosed before laparotomy, unless scintiscan of the abdomen is performed (p. 210).

The etiology of thyroid aplasia or ectopism is not known. The question as to whether maternal thyroid antibodies which cross the placenta may damage the fetal thyroid is a matter of debate (BLIZZARD, 1960; CHANDLER, 1962). Most mothers of athyrotic children have no thyroid antibodies, and most mothers with thyroid antibodies have normal children. Thyroid aplasia and hypoplasia occur sporadically in most cases and only very rarely is there a familial incidence (CHILDS, 1954; BERNHEIM, 1961). The mothers of athyrotic children usually have a normal thyroid function, but there is an increased incidence of hypo- and hyperthyroidism in their families. Both sexes are equally affected and it has not been possible to incriminate exposure to toxins and infectious agents (toxoplasmosis) as a cause (ANDERSEN, 1969).

A few cases of hypothyroidism acquired during fetal life due to radioiodine treatment of the mother have been reported (FISHER,

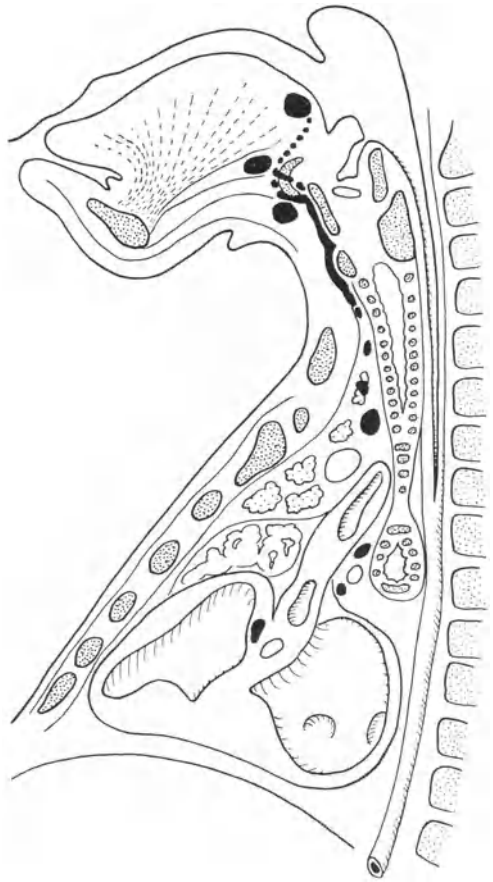


Fig. 7. Schematic representation of the most frequent locations of dystopic thyroid glands. (From S. C. WERNER: The thyroid, New York 1955)

1963). Treatment of the mother with antithyroid drugs may produce fetal hypothyroidism and goiter. Both are rapidly reversible after birth, but it is not known whether the episode of fetal hypothyroidism leaves traces in later life in the form of subtle mental retardation. Treatment of the mother with pharmacological doses of iodide, e.g. for asthma, may produce fetal hypothyroidism and goiter. The condition has a serious prognosis (CARSWELL, 1970).

2. Acquired Childhood Hypothyroidism

Hypothyroidism of the newborn due to maternal drug treatment has been discussed above. Hypothyroidism due to drug treatment of the child itself is very rare, due to the rarity of thyrotoxicosis in childhood. Two causes should be considered when hypothyroidism develops in a previously healthy child. The first is the failing function of a hitherto unrecognized ectopic and/or hypoplastic thyroid gland. This is reported to occur frequently during puberty. The second cause is idiopathic hypothyroidism due to spontaneous atrophy of the thyroid gland, also called chronic thyroiditis, atrophic variant (WINTER, 1966). The pathogenesis of this disease, which is probably autoimmune in nature, has been discussed with that of adult hypothyroidism.

Cystinosis, a rare generalized metabolic disorder which usually leads to death in renal insufficiency, has recently been found to cause hypothyroidism. Four out of 16 patients studied by CHAN (1970) were frankly hypothyroid. Autopsy revealed severe thyroid atrophy with cystine crystals in epithelial cells.

The differentiation of acquired hypothyroidism from the congenital form is not usually difficult. In the acquired form, growth and mental development are normal during the first years of life and the signs and symptoms are similar to those of the disease in adulthood. Slowing of mental functions is present, but the mental retardation is much less pronounced. Irregularities in the bones and body proportions are also less conspicuous than in the congenital form.

3. Genetically Determined Defects of Thyroid Hormone Biosynthesis or Action

Thyroid hormone biosynthesis and action occur in a sequence of several steps (p. 139), each of which may theoretically be the site of an hereditary defect. The defects known to date are listed in Table 4. An exhaustive review on the subject has been written by STANBURY (1972), and it is his classification which is used in this discussion. All the defects known so far are of the autosomal recessive type. Consanguinity of the parents is often demonstrated and siblings are frequently affected.

The clinical picture is very similar in most defects, with the exception that peroxidase deficiency is often associated with nerve deafness (Pendred syndrome, see below). Goiter is occasionally present at birth, but usually develops during early childhood. It becomes very large and can no longer be overlooked by adolescence. Hypothyroidism may be mild or marked, but is rarely as severe as in congenital aplasia of the thyroid. The symptoms and signs are the same as for the other forms of childhood hypothyroidism. It can safely be said that the occurrence of a large goiter in a hypothyroid child is almost diagnostic of one of the hereditary defects.

Pathological examination of the thyroid gland usually shows a multinodular goiter with all the histological signs of intense stimulation by TSH, i.e. very sparse colloid with a micro-

follicular picture. Trabecular epithelial parts are also found. Polymorphism of epithelial cell nuclei is often encountered, and sometimes the picture might be interpreted as that of a malignant tumor, but metastases are practically never seen.

a) Deficient Active Iodide Transport (STANBURY, 1960; WOLFF, 1964)

This is a rare cause of hereditary hypothyroidism. The thyroid is unable to accumulate iodide from the blood in this condition, and the defect can be imitated pharmacologically by the administration of perchlorate, a drug that competitively inhibits active iodide transport (p. 140). MEDEIROS (1972) has described two patients with a partial defect. There is no complete biochemical explanation for the defect as yet. It is konwn that active iodide transport requires energy supplied by a membrane-bound Na^+- or K^+-activated ATPase. However, it is unlikely that the latter enzyme is absent, since this is hardly compatible with cell survival. The salivary glands, which normally also actively accumulate iodide are also affected. The diagnosis is made from a lack of thyroidal accumulation of radioactive iodide and by the low PB ^{131}I level in the serum after a dose of radioiodide; it is confirmed by a lack of radioiodide accumulation in saliva when compared to the plasma concentration and finally by *in-vitro* incubation of excised thyroid tissue. GILBOA (1963) has clearly shown that the subsequent steps of thyroid hormone synthesis are intact. He administered large doses of iodide to a patient, which raised the plasma iodide to such levels that enough iodide penetrated into the thyroid gland by passive diffusion to make active transport unnecessary. Production of normal amounts of thyroid hormone was thus possible and the patient became euthyroid. However this is not the recommended routine treatment, since overdosage of iodide led to hyperthyroidism in this case.

b) Defective Thyroglobulin Iodination (Iodine Organification) and Pendred Syndrome

Many patients with this defect have been reported since it was originally described by STANBURY (1950). It now appears that this is a heterogeneous group of disorders. The two features common to all cases are the occurrence of goiter and the fact that radioiodine accumulated by the thyroid gland can be discharged by the administration of perchlorate (see p. 243). With the elucidation of the steps necessary for iodination of thyroglobulin (p. 140) it has become customary to describe the disease as due to deficiency of thyroidal peroxidase, the enzyme presumably necessary for the oxidation and organification of iodine. However, as will be seen below, the term peroxidase deficiency does not cover all defects described in this disorder, and should therefore be used with caution.

HAGEN (1971) has classified patients with defects in iodide organification as follows: in *type A* thyroid peroxidase is completely absent (VALENTA, 1973). The patients are severely hypothyroid cretins with a very low plasma hormone level. Administration of perchlorate leads to discharge of all accumulated iodide. In *type B* (Pendred's syndrome) the patients are euthyroid (MILUTINOVIC, 1969), have a goiter and suffer from nerve deafness. Perchlorate discharges part of the radioiodide and peroxidase activity is normal (LJUNGGREN, 1973; BURROW, 1973). *Type-C* patients are euthyroid, have a goiter, a partial perchlorate discharge and abnormal peroxidase. Enzyme activity is absent *in vitro* when tested in normal conditions, but the addition of hematin, the alleged prosthetic group of the enzyme, partially restores enzyme function. A defect in the binding of the prosthetic group to the apoenzyme has been postulated (HAGEN, 1971; NIEPOMNISZCE, 1972 and 1973).

REINWEIN (1970) has described a case of defective iodide organification where the thyroid predominantly secreted triiodothyronine. It has not been established to which of the above classes his patient belongs. It is possible that predominant triiodothyronine synthesis is merely a consequence of the intense TSH stimulation and the low degree of thyroglobulin iodination, which are necessarily present in all hypothyroid patients. This leads to a preferential synthesis of monoiodotyrosine and consequently of triiodothyronine. BELLABARBA (1972) has described 4 patients with almost selective triiodothyronine secretion, but the nature of the underlying biochemical defect was not specified.

The cause of deafness in Pendred's syndrome has not yet been established.

c) Failure of Iodotyrosine Coupling

The last step in the biosynthesis of thyroxine is the coupling of two diiodotyrosyl residues of thyroglobulin to form thyroxine. Details of this reaction are not yet fully understood (p. 141). Several patients showing good evidence for the failure of iodotyrosine coupling have been described. They all had a goiter, were moderately or borderline hypothyroid and had a high thyroidal radioiodine uptake (STANBURY, 1963). A large fraction of the radioiodide taken up by the thyroid was rapidly lost again in non-

hormonal form. SHIMAOKA (1972) recently described a case with failure of iodotyrosine coupling who secreted the unusual iodocompound diiodo-p-hydroxyphenyl lactic acid, an alleged intermediate in the coupling reaction. It remains to be seen whether this compound is found in other cases.

Diagnosis of coupling failure is based mainly on the exclusion of other defects and on the demonstration of labeled mono- and diiodotyrosine but not of thyroxine in thyroid tissue excised after the administration of radioiodine. This pattern of iodine distribution can also be simulated by iodine deficiency, which must therefore be excluded by measurement of thyroidal and urinary ^{127}I content.

d) Failure of Iodotyrosine Deiodination

A normal molecule of thyroglobulin contains about 2 residues of thyroxine and 10 to 15 residues of mono- and diiodotyrosine. When thyroxine is to be released the whole thyroglobulin molecule is hydrolyzed into its constituent amino acids. Mono- and diiodotyrosine, neither of which has any hormonal activity, are immediately deiodinated within the gland and most of the iodide thus liberated is reutilized for thyroglobulin iodination without leaving the gland (p. 142). If the iodotyrosines are not deiodinated their iodine cannot be reutilized and they diffuse out of the gland and are excreted in the urine. A number of patients meeting the criteria for a defect of deiodination have been described (MCGIRR, 1959). All of them had a goiter, were either frankly or borderline hypothyroid, and were mentally retarded. After a dose of radioiodine the thyroidal uptake was high and turnover of the isotope was rapid. Labeled iodotyrosines, normally absent, were found in the urine. The diagnosis is confirmed by the administration of ^{131}I-labeled mono- or diiodotyrosine. Normally only traces of the label appear in the urine in the form of the injected compound and most of the urinary ^{131}I is iodide, presumably because the deiodinating enzyme is also present in various extrathyroidal tissues including the liver. STANBURY (1972) has presented presumptive evidence that heterozygotes can also be detected with this test. NAKAJIAMA (1971) has recently described patients in whom he thought that diiodotyrosine deiodination was deficient while that of monoiodotyrosine was normal. The defect was thought to be transmitted as an autosomal dominant, in contrast to the findings in all other sibships reported so far.

Since severe dietary iodine deficiency can cause some of the same clinical findings and radioiodine turnover values, this condition has to be carefully excluded by measurement of urinary ^{127}I before the diagnosis of failure of iodotyrosine deiodination can be accepted.

The reason why these patients are hypothyroid is not immediately apparent. All the steps of hormone biosynthesis and release are in fact intact and the defect is located in a side reaction of iodine metabolism. This side reaction is, however, very important for the preservation of iodine stores, and when it is impaired the gland suffers severe iodine deficiency. The accuracy of this interpretation was clearly demonstrated by VAGUE (1962), who treated patients with Lugol's solution. The increased iodine supply led to a complete normalization of thyroid function, confirming that hormone biosynthesis and release were proceeding normally.

e) Diminished or Altered Thyroglobulin Synthesis

A number of case reports have appeared where a defect in thyroglobulin synthesis was postulated (see STANBURY, 1972, for review). Some of the patients had a thyroglobulin with altered physicochemical properties, while others produced no distinct iodoprotein at all, and still others synthesized and iodinated albumin, which in normal thyroids is present in only small amounts. Two cases of complete absence of thyroglobulin biosynthesis have recently been well documented by LISSITZKY (1973). This group of defects thus does not appear to be homogeneous with regard to the basic defect. Clinically the patients are hypothyroid or borderline euthyroid and have a goiter. The radioiodine uptake is high. The diagnosis is made by biochemical examination of excised thyroid tissue. It must be borne in mind that any condition that leads to intense and prolonged TSH stimulation of the thyroid gland, e.g. iodine deficiency or other inborn defects of hormone synthesis, will produce a hyperplastic goiter depleted of colloid and thyroglobulin. The causes of thyroglobulin depletion mentioned above therefore have to be rigorously excluded before the diagnosis of deficient thyroglobulin synthesis can be accepted. In a rat goiter MONACO (1973) has ascribed the lack of thyroglobulin synthesis to a defect in one of the enzymes necessary for the addition of carbohydrate to the protein. It is conceivable that similar defects occur in man.

f) Abnormal Iodinated Serum Peptides

Several patients with hypothyroidism, mental retardation, goiter, and a high level of protein-bound iodine have been detected. Analysis of their serum iodine has revealed that a large part is not extractable into butanol (thyroxine is extracted almost 100% into butanol). The

abnormal iodinated substances were identified as peptides which migrated with albumin on paper electrophoresis. Hydrolysis of these peptides with trypsin yielded mono- and diiodotyrosine. Such iodinated peptides are also present in the serum in chronic lymphocytic thyroiditis, in thyroid cancer, and occasionally in endemic goiter. The basic biochemical defect and the mode of inheritance are not known (DEGROOT, 1958; WERNER, 1960; LAMBERG, 1963; STANBURY, 1972).

g) Rare Inborn Errors

STANBURY (1968) described an 8-year-old congenitally hypothyroid patient with no goiter whose thyroid failed to respond to TSH injected *in vivo* and also to TSH added *in vitro*. He postulated a defect in thyroid responsiveness to TSH, but other interpretations of the findings are also possible.

REFETOFF (1967, 1972) investigated families in which several members had small goiters, were borderline hypothyroid, had nerve deafness, and, surprisingly, had a high circulating thyroid hormone level. He thought the syndrome was due to an end-organ resistance to thyroid hormone action. A characteristic radiologic findings was stippled femoral epiphyses. Basically similar cases were observed by LAMBERG (1973) and BODE (1973).

REINWEIN (1963, 1964) reported a case of goiter with deaf-mutism where the synthesis of thyroxine was normal, but the release of the hormone from thyroglobulin was impaired. Biochemical examination of the gland suggested that the condition was probably due to a deficiency of thyroid protease.

4. Secondary Hypothyroidism in Childhood

The causes of secondary hypothyroidism in childhood are listed in Table 4. It rarely occurs as an isolated TSH deficiency and is most frequently associated with growth hormone deficiency in the syndrome of pituitary dwarfism (p. 98ff.). The availability of synthetic thyrotropin releasing hormone has made it possible to show that in the majority of cases the disease is of hypothalamic rather than pituitary origin (p. 160).

5. Clinical Features of Childhood Hypothyroidism

a) Features in the Neonate

The success of substitution therapy is dependent mainly on early diagnosis of hypothyroidism in the neonate, which justifies special attention to the early signs. The athyrotic or hypothyroid neonate usually shows none of the classic clinical features of hypothyroidism, and the differential diagnosis against Down's syndrome may be very difficult (p. 176). That an athyrotic neonate suffered from hypothyroidism during fetal life is often documented by two facts: first, the bone age is slightly retarded at birth; secondly, mental retardation may become manifest in later life, even when thyroid hormone replacement is started immediately after birth. Both facts support the view that maternal thyroid hormones cross the placenta in insufficient amounts and that the fetus is therefore dependent on its own hormone supply.

Pregnancy is sometimes prolonged in the case of a hypothyroid child. Weight at birth is often slightly above average. Body length at birth may be less than normal, normal, or even above normal. Prenatal bone maturation is sometimes retarded, which is easily demonstrated by the lack of calcification in the distal femoral epiphysis at birth. Occasionally a large tongue and a puffy red face resembling that in Cushing's disease is noted. Very often the physiologic neonate icterus is prolonged (icterus prolongatus).

In the first few weeks of life the first symptoms of diminished metabolism become apparent. The baby is conspicuously quiet, needs a lot of sleep, becomes a poor nurser and is constipated. He is unusually good-tempered, rarely cries and lies still in bed. In spite of the feeding problem the baby looks well-nourished (Fig. 8). Gradually, physical and mental retardation with motor inactivity, flaccidity of the musculature, protrusion of the abdomen (often associated with umbilical hernia), and the yellow, dry and thickened skin become increasingly apparent. The baby does not kick, and smiles and lifts his head later than normal. His face becomes puffy and the tongue is enlarged (Fig. 8). The hair is dry and grows slowly. The neck is usually too fat to permit proper palpation of the so-called "bare trachea" in thyroid aplasia. Temperature and pulse rate tend to be low. All these symptoms are sufficiently developed by the second month to raise the suspicion of hypothyroidism in the experienced doctor.

b) Features in Early Childhood

In the second six months of life the symptoms become so marked that even mild cases can be readily diagnosed. All symptoms gradually become worse in subsequent years.

The mental and physical development of these children is greatly retarded. They are unusually peaceful, sometimes almost stuporous. All their actions are extremely slow. They are severely constipated. Apart from the skin changes, which will be discussed later, physical exmination reveals striking abnormalities. The head is large and brachycephalic. The facies is broad and puffy and the features are coarse. The expression is dull and content. The forehead is low and covered by thick wrinkles. The nasal bridge is broad and flat and the

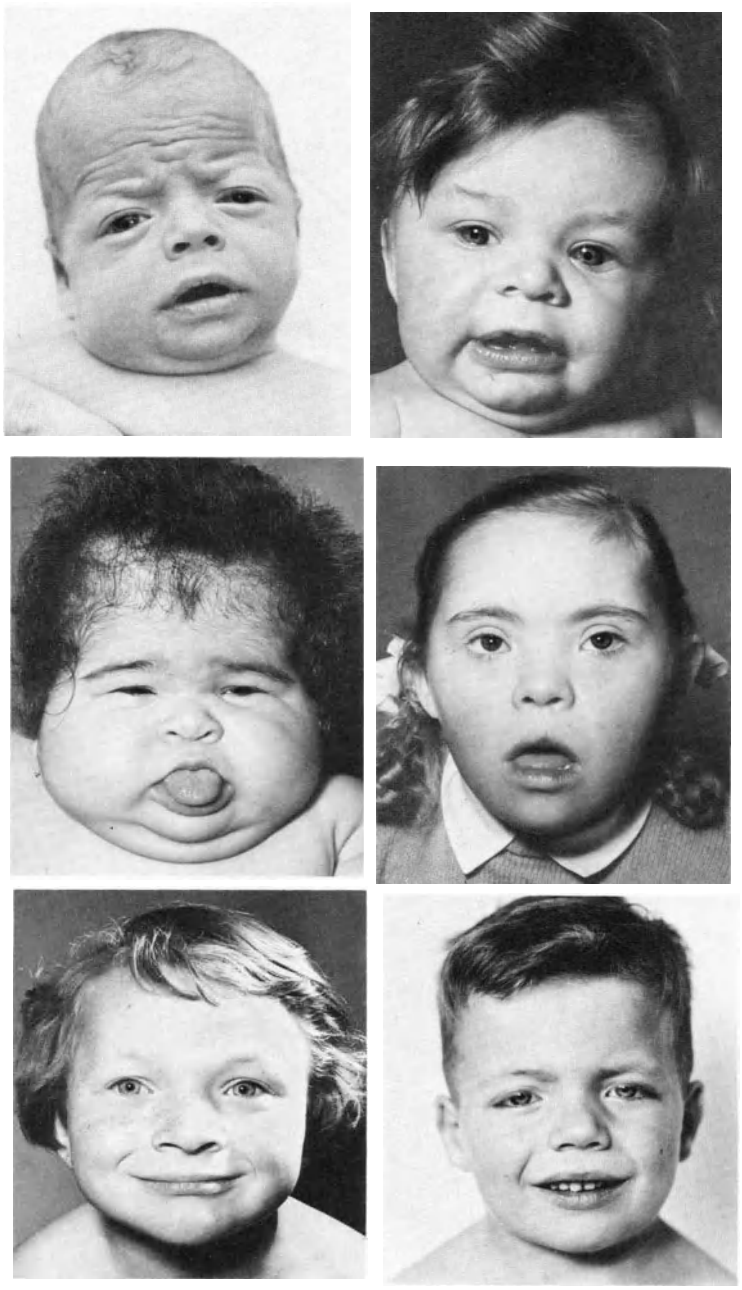


Fig. 8. Facial expression in congenital hypothyroidism and in Down's syndrome in childhood. Top left: age 2 months, untreated thyroid aplasia. Top right: age 5 months, untreated moderate hypothyroidism. Middle left: age 2 years, thyroid aplasia, untreated. Middle right: age 7 years, Down's syndrome. Bottom left: age 7 years, hypothyroidism, on insufficient replacement therapy. Bottom right: age 7 years, thyroid aplasia, on insufficient replacement therapy. Note the enormous difference between thyroid aplasia and moderate hypothyroidism in babyhood (top figures). At age 7 the facial expression in insufficiently treated hypothyroidism is not strikingly abnormal, but still shows the round face and the broad features. Note also the difference between hypothyroidism and Down's syndrome. In contrast to hypothyroidism Down's syndrome causes slanted eyes and an epicanthal fold. The mouth is also enlarged and but not to the same degree as in hypothyroidism

nostrils point forewards instead of downwards. The interocular distance is increased. The mouth is large and the lips and tongue are thickened and swollen (Fig. 8). Dental eruption is delayed. Defects in dental enamel are frequent, as is dental caries. The hair is dry. Speech is slow and badly articulated and the voice is hoarse. Deafness may later be noted in Pendred's syndrome (p. 168). Occasionally the trachea can be palpated despite a fat neck, and there is an impression that there is no overlying thyroid tissue ("bare trachea"), but this, of course, is an unreliable sign for the absence of the thyroid.

The body is not only short, but also fat and plump. The extremities are short and the abdomen protruding.

c) The Specific Symptoms of Developmental Retardation

Thyroid insufficiency, even of a slight degree, causes marked retardation of physical and mental development. This is particularly true in children with thyroid aplasia, who at the age of 10 years may physically be at the stage of babyhood, if they have never been treated. They rarely reach the age of 20 and die much earlier in most cases.

In hypothyroidism the retardation of growth is characterized mainly by delayed bone development and maturation, and delayed development of body proportions. The body proportions as measured by the length of the upper segment (symphysis to head), the lower segment (symphysis to feet) and the span (fingertips to fingertips with extended arms) remain infantile (cf. Chap. XIX), i.e. the extremities are too short (Figs. 10, 13, 14). This is in contrast to dwarfism from other causes. The head is too large in relation to the body and retains its infantile shape. This results in the typical brachycephalic head with coarse facial features.

Fig. 9 shows growth, weight gain, bone development and intelligence in a case of severe hypothyroidism, expressed in terms of developmental age correlated with chronological age (Chap. XIX). As a rule, bone age is more and weight age (due to slight obesity) less retarded than height age. The dental age is not shown. It is usually less retarded than the other parameters, although a delay in dentition is almost always demonstrable. Intelligence age varies widely from case to case, but rarely exceeds height age.

If all the parameters discussed are considered together hypothyroidism produces a characteristic developmental type after the age of early babyhood (Fig. 10). This type again

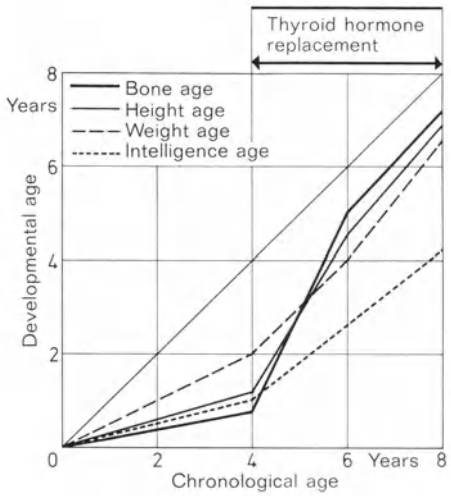


Fig. 9. Schematic representation of growth, weight gain, development of bones and intelligence in a case of severe congenital hypothyroidism. Treatment was started at age 4 (KspZ)

changes in a characteristic fashion upon optimal thyroid substitution therapy (Fig. 9). Bone age is accelerated more than weight age, and mental development will ultimately be least influenced by thyroid hormone treatment (p. 176). In untreated cases puberty is delayed in

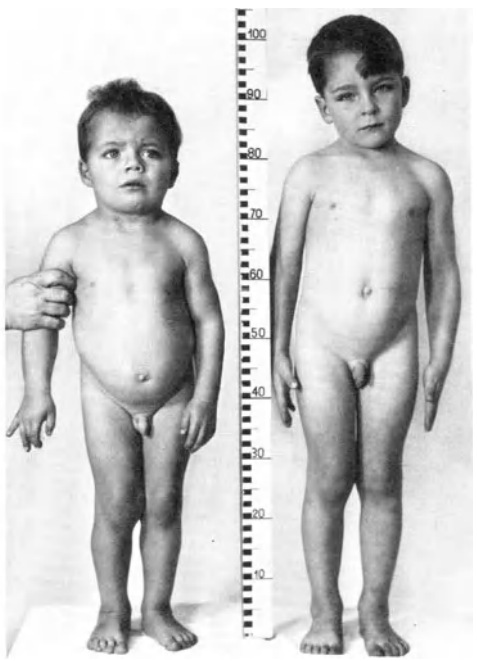


Fig. 10. Twins, age 4½ years. The boy on the left suffers from inadequately treated thyroid aplasia, the one on the right is healthy. Note the short stature, the protruding abdomen, the large brachycephalic head, the broad coarse face, the thick neck, and the infantile proportions. In the affected child the ratio of the upper to the lower segment is 1.4:1, in the healthy twin it is 1.26:1

accordance with the delayed bone age. Sometimes it is incomplete or fails to occur at all. (The relationship between puberty and bone age is discussed in Chap. XIX.)

d) Skeletal Changes

The slow longitudinal growth of long bones (short extremities) and the retardation of bone development have already been mentioned. This retardation is apparent in delayed appearance of ossification centers, fontanelle closure, pneumatization of skull bones, and epiphyseal closure. An X-ray of the hands should always be made for determination of the bone age (Chap. XIX); in addition to the delayed apperance of ossification centers the plate will show other typical features, such as the intensively calcified preparatory zone of calcification at the ends of the diaphyses (Fig. 11). Substitution therapy causes bone growth, but this zone remains visible for a long time as a densely calcified transversal "growth line" (Fig. 11). Corresponding circular lines will be visible in the ossification centers. This intensive calcification of bone has been attributed to the slow growth of the bone matrix due to reduced osteoblast activity (low alkaline phosphatase) together with a relatively good supply of calcium. This leads both to an intense calcification of bone and to a reduction in intestinal calcium absorption, as is documented by the low urinary calcium excretion. This explains why rickets, i.e. diminished calcification of the bone matrix, virtually never occurs in hypothyroidism, and also why vitamin-D intoxication is easily produced. These changes probably also cause the osteosclerotic changes and the soft tissue calcifications occasionally observed in untreated patients with thyroid aplasia.

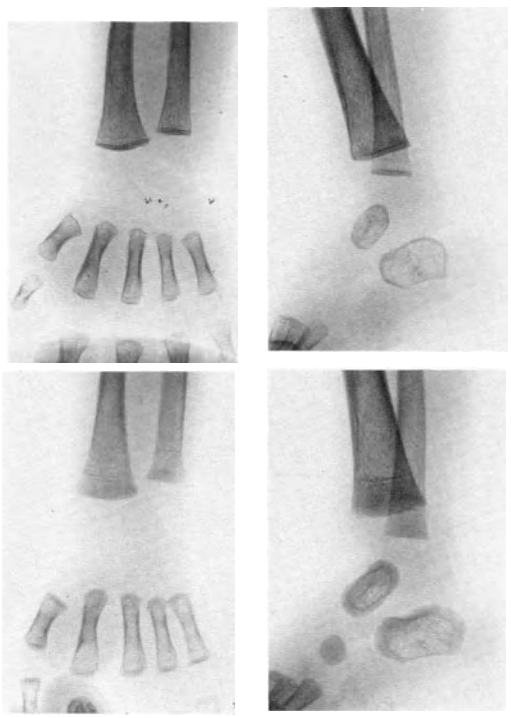


Fig. 11. X-rays of hands and feet in a case of congenital hypothyroidism. Top: At age 9 months, just prior to treatment. Bottom: At age 11 months, after two months of treatment. The phase of slow growth preceding treatment can still be recognized after treatment from the dense transversal line in the long bones and from the dense circular line in the talus and calcaneus (KspZ)

Another typical skeletal change is the irregular multicentric calcification of ossification centers, the so-called epiphyseal dysgenesis or "cretinoid osteochondropathy". This sign becomes particularly marked during intense substitution therapy, which produces a rapid calcification of the epiphyses. It can be observed in all epiphyses and ossification centers, but is most typical in the femoral head. This radiologic finding is very similar to that in Perthes' disease, but differs from it in always being bilateral. The femoral head becomes completely calcified during therapy, but severe deformity with coxa vara always persists (so-called cretinous hip, Figs. 12, 31, p. 220). The hip deformities contribute to the clumsy gait of most congenitally hypothyroid patients.

Localized bone changes occur in the spine in the form of thoracal kyphosis with wedge-shaped compression of the second lumbar vertebra. The sella turcica is often enlarged, probably due to hyperplasia of the anterior pituitary (increased TSH secretion, see p. 155).

e) Skin Changes

As in cases of adult hypothyroidism, the skin has a yellowish to dirty brown color. It feels thickened, rough and dry. In older children grayish, dirty-looking hyperkeratotic knees are often seen. The skin of the extremities is not only cool, but sometimes frankly cyanotic. Histologic examination reveals increased metachromasia and increased numbers of mast cells.

The subcutaneous fat is usually well developed, but of a peculiarly flaccid firmness. In severe cases of hypothyroidism striking collar-like supraclavicular fat deposits develop (Fig. 13).

Scalp hair is sparse, dry and thickened (Fig. 8). After the age of two years, marked hypertrichosis with lanugo-like hair on the back and on the extensor surfaces of the extremities often develops, pubic and axillary hair remaining absent until puberty.

All changes in the skin, hair and subcutaneous fat are reversed by substitution therapy.

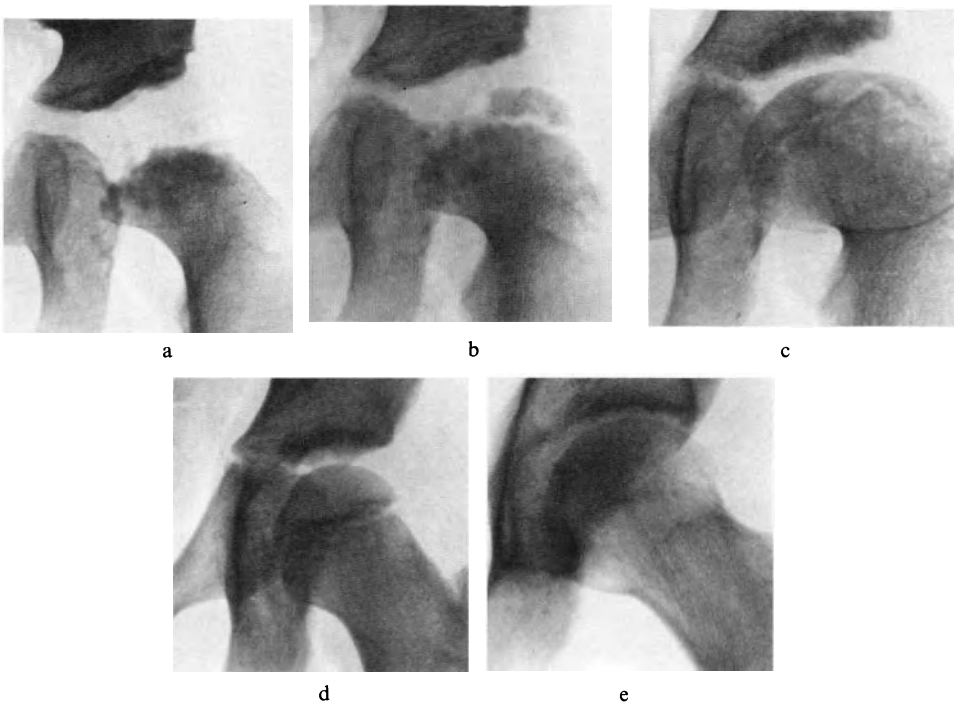


Fig. 12. a to c) Hip joint of a boy with congenital hypothyroidism at age 12, 14, and 17 years. Thyroid hormone treatment was instituted shortly after the first picture. d, e) Hip joint of healthy boys at age 11 and 14 for comparison. Note the very deficient ossification which rapidly improves after treatment, but still remains irregular. The femoral head is grossly deformed (KspZ)

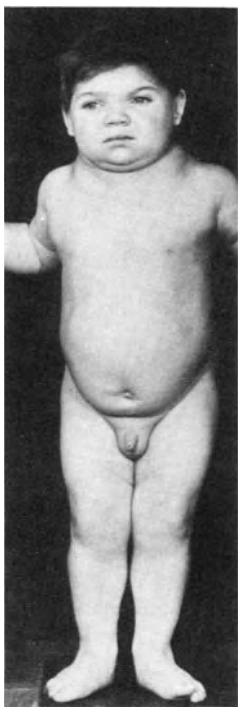


Fig. 13. Patient with congenital thyroid aplasia at age 22 years. The height is 105 cm. Note the typical facial expression, the developmental retardation and the collar-like supraclavicular fat. (Prof. E. MARTIN, Prof. B. COURVOISIER, Policlinique médicale de l'université de Genève)

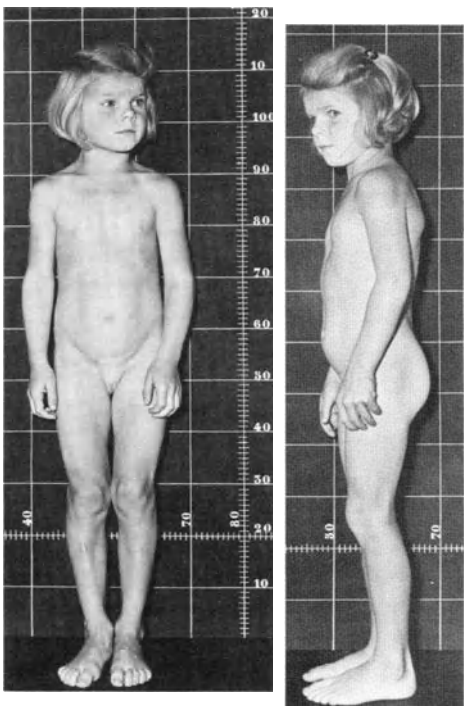


Fig. 14. Congenital hypothyroidism, inadequately treated. The girl, age 8 years, has a mental and physical development corresponding to a 4-year-old. Note the typical facial expression and spastic stature with slightly flexed extremities

f) Neuromuscular System

In the infant and the young child the musculature is usually underdeveloped and flaccid. This explains the large, protruding abdomen. In the older child, and occasionally in the infant, there is generalized muscle hypertrophy with muscular rigor. The result is an almost athletic appearance associated with clumsy movements. In the rare cases of the Kocher-Debré-Sémélaigne syndrome, muscular hypertrophy dominates the clinical picture (DEBRÉ, 1935; CROSS, 1968; MEDEIROS, 1972). All movements are slow and clumsy. The patients keep their stiff limbs in a slightly flexed position (Fig. 14). They stand and walk with flexed knees and with their feet turned in. There is a peculiar lack of spontaneous involuntary movements. Tendon reflexes are very active, but the relaxation phase is prolonged. A pseudomyotonic reaction is often elicited by tapping the muscles (p. 158).

The electroencephalogram shows slowed activity with diminished potentials. Even the treated patient may have general dysrhythmic brain activity which parallels the irreversible mental retardation to a certain degree.

g) Cardiovascular System and Blood

The pulse rate is low and blood pressure is reduced with few exceptions. The heart is occasionally enlarged (myxedematous heart, see p. 156), which is attributed partly to myxedematous changes within the myocardium and partly to concomitant anemia. The electrocardiogram is often normal. Occasionally the same changes are seen as in the adult (low voltage, see p. 156).

Anemia is almost always present. As in the adult (p. 157) it may be either hypochromic or hyperchromic. A constant finding is hypoplastic hypocellular bone marrow. Serum iron may be elevated due to diminished incorporation into hemoglobin. The hyperchromic anemia, which responds only to thyroid hormone substitution, is probably more characteristic of hypothyroidism, while hypochromic anemia is rather nonspecific and may be related to alimentary iron deficiency. The white blood picture may be normal or show lymphocytosis. True juvenile pernicious anemia has been reported to coexist with childhood hypothyroidism (OLIVER, 1969).

h) Metabolic Changes

A lowered basal metabolic rate is as characteristic as in adult myxedema. Due to technical difficulties and uncertainty about normal values in children with dwarfism, the basal metabolic rate is of no great clinical importance.

In contrast, the measurement of the serum thyroxine concentration and radioiodine uptake studies are as important in children as in adults. Normal values are very comparable to those of adults.

As in the adult, an increase in beta-lipoproteins, with hypercholesterolemia and hypercarotenemia, is present in older children. Hypercholesterolemia is absent, however, in most babies and is infrequent in small children. On the other hand, the serum alkaline phosphatase is often diminished in the small child (normal values for children: 30 to 140 IU). This is due mainly to decreased osteoblast activity, and possibly also to hepatic changes. Calcium and phosphate in the serum are usually normal. Rare cases have shown hypercalcemia with osteosclerosis (ROYER, 1954).

Urinary excretion of creatine and calcium are reduced.

Urinary steroid excretion and tests of adrenal cortical function may indicate slight secondary adrenocortical insufficiency. The cortisol secretion rate is diminished (KENNY, 1967). Growth hormone secretion shows a less pronounced rise in response to hypoglycemia. These findings are comparable to those in pituitary dwarfism, but differ in that they are reversible by thyroid hormone substitution.

Occasionally pubertas praecox occurs (VAN WYK, 1960), which has been ascribed to an overlap between the feedback of gonadotropins and TSH. In girls this may be accompanied by galactorrhea (p. 158) with elevated prolactin levels. Thyroid hormone substitution reverses the signs of early puberty.

i) Differential Diagnosis

After infancy, untreated cases of athyroidism and hypothyroidism are immediately and easily recognized. The cretinoid features, physical and mental retardation, characteristic skeletal proportions, skin changes, slow pulse, and low blood pressure all combine to give an unmistakable picture. Of course the clinical spectrum reaches from complete athyroidism to mild failure, according to the nature of the thyroidal defect. Nonetheless, mental and physical retardation is a mandatory sign, even in mild cases. Hypothyroidism must therefore be suspected in every case of dwarfism and mental retardation.

Severe untreated cases are now rarely encountered later than babyhood, because the correct diagnosis is usually made. However,

untreated babies and badly treated older children are still frequently encountered.

Measurement of the ankle reflex time is a simple and reliable procedure. In hypothyroidism the reflexes are definitely prolonged, but it must be borne in mind that normal values are lower in children than in adults (p. 241).

Most important for the establishment of the diagnosis in small babies is the measurement of serum hormone concentrations and of the radioiodine uptake. If the values are not clear-cut and the suspicion of hypothyroidism persists, a TSH stimulation test should be performed, and if possible also a TRH stimulation test (p. 242). A TSH test must also be performed in cases where thyroidal ^{131}I uptake is already suppressed due to thyroxine treatment. If the stimulation tests cannot be performed in doubtful cases, one must resort to establishing the diagnosis from the failure or success of thyroid hormone substitution therapy given over several weeks. A clear-cut improvement of signs takes place only in hypothyroidism.

In babies, hypothyroidism is often mistaken for rickets. Growth is retarded in both diseases, but otherwise signs are completely different, in particular the bone changes seen on X-ray and alkaline phosphatase in the serum (elevated in rickets, lowered in hypothyroidism).

Chondrodystrophy and gargoylism (Hurler's syndrome) are also occasionally confused with hypothyroidism, the signs they have in common being dwarfism and a large head with coarse features. In chondrodystrophy the extremities are short, as they are in hypothyroidism, and gargoylism is often associated with mental retardation. In both diseases, however, all other features of hypothyroidism in particular the cutaneous and cardiovascular changes and the metabolic abnormalities, are absent.

For the differential diagnosis of dwarfism the reader is referred to Chap. XIX.

The differential diagnosis is also difficult in cases with marked mental retardation and some degree of physical retardation but without the symptoms of severe hypothyroidism. Some disorders due to brain damage and several metabolic diseases have to be considered in these cases (phenylketonuria and others). For the inexperienced doctor the differential diagnosis from Down's syndrome often proves very difficult. In both conditions patients have a dull facial expression with plump features, a wide mouth and a large, protruding tongue. The epicanthal fold and the slanted eyes, however, distinguish Down's syndrome from hypothyroidism (Fig. 8). Moreover, in Down's syndrome these are already present at birth, whereas in hypothyroidism the development of the typical facies takes several weeks. Muscular hypotonia, short stature, constipation, hoarse voice, and mental retardation in Down's syndrome may contribute to the confusion with hypothyroidism, but the motor inactivity, the skin changes and the cardiovascular changes are absent. On the contrary, children with Down's syndrome are often hyperactive and agitated. Another distinctive sign is the high frequency of congenital heart disease in Down's syndrome and its rarity in hypothyroidism. Occasionally both diseases are found simultaneously in one patient (HARRIS, 1967). In such cases the typical hypothyroid signs have been found in addition to the diagnostic chromosomal changes (trisomy 21).

6. Treatment and Prognosis

Treatment of childhood hypothyroidism must be by full oral hormone substitution. In acquired hypothyroidism the aim of normal physical and mental development is more easily achieved than in congenital hypothyroidism, where normal physical and mental development is only possible if substitution therapy is begun within the first months of life and consistently maintained in an adequate dosage throughout the growth period. Delayed institution of therapy, interruptions, or inadequate dosage will seriously compromise the final result. The following guidelines are therefore meant primarily for the treatment of congenital hypothyroidism. A change of doctor is often detrimental to the patient. The new doctor will find no signs of hypothyroidism if the child has been properly treated, and may consider treatment unnecessary. Months often pass before the developmental retardation becomes manifest and the error is recognized. Such interruptions are deleterious for the development of a child and are to be avoided at all costs. In case of doubt about the original diagnosis it is wise to continue treatment until the previous patient data are obtained or until the diagnosis is confirmed by a TSH test (p. 242).

Unfortunately, even an early diagnosis and correct continous treatment do not always guarantee normal mental development. As in endemic cretinism, this must be attributed to prenatal brain damage which is incompletely reversible. If treatment is started late or not continued consistently a clear improvement can be observed, but never a full normalization of development. Unfortunately, most patients with congenital hypothyroidism who reach adulthood belong to the latter group. Basal metabolic rate,

pulse, blood pressure and serum lipids have become normal, and constipation and skin symptoms have been corrected. Nontheless, short stature (Fig. 10), a plump broad facies (Fig. 8), general slowness, decreased fitness, slow and clumsy speach and varying degrees of mental retardation persist. Puberty is delayed, but otherwise normal. Only extremely few patients have a completely normal mental and physical development fitting them for normal schooling and professional training.

Experience has shown that years of treatment are needed to catch up with normal growth and that normal mental development can only be assured by early and regular treatment with proper dosage (Fig. 9). Therefore substitution therapy *during the whole of the growth period, in particular in infancy, is given at the highest dose that can be tolerated.* This principle is abandoned only in children in whom treatment was started late; when the doctor is convinced, on the basis of the results of several months of full treatment, that complete normalization of mental development cannot be achieved, the dose may then be reduced if the patient becomes more placid and more easily manageable. After the age of 10, athyrotic cretins who have never been treated and have remained at the stage of an infant are best left without therapy, since their mental retardation can no longer be improved. Thyroxine treatment in such cases can convert a passive, quiet patient into an agressive, restless one who needs continuous supervision.

The preparation of choice for the treatment of childhood hypothyroidism is L-thyroxine. Some doctors still use the time-honored desiccated thyroid, which is difficult to standardize, while others prefer a mixture of L-thyroxine and triiodo-L-thyronine. As outlined in a previous section (p. 164) this mixture has no real advantages. For a 9-year-old the average dose is 200 µg thyroxine, or 200 mg desiccated thyroid. The dose for a two-year-old is about one half, for an infant about one third and for a neonate about one fifth of the above amount. The usual procedure is to start with half the estimated dose and increase to full substitution after 2 to 3 weeks. The medication is given in one dose per day. The dose is adapted for each case from the above values by trial and error. The correct dose is the highest one tolerated without signs of hyperthyroidism such as agitation, restlessness, tremor, tachycardia, perspiration, insomnia and diarrhea. Correct dosage produces normal physical activity and normal body functions. In infants symptoms of hypothyroidism disappear within a few weeks and lethargy, lack of appetite, skin changes, pulse and constipation are greatly improved. An initial weight loss is regularly seen. Hair often falls out but grows again rapidly without the previous thickening and dryness. Growth and physical development become normal. After a delayed onset of therapy, an accelerated "catch-up" growth is often seen. The ankle reflex time is a good index of the effects of substitution therapy, providing age-adjusted normal values are used (p. 241). The serum total thyroxine can also be helpful, (cf. p. 165). The measurement of serum cholesterol is less recommended as a guide to therapy. Growth and bone age should be monitored at six-monthly or yearly intervals. With proper substitution therapy instituted early, both parameters progress normally. Bone age may sometimes get ahead of growth. If this tendency becomes marked, early epiphyseal closure may occur. To prevent this, the dosage is slightly reduced.

In the relatively rare cases of hereditary defects of hormone biosynthesis it is often necessary to resort to total thyroidectomy because of pressure symptoms and because of the danger of malignancy in the huge nodular goiter. In cases with only small goiters, thyroxine substitution therapy alone is sufficient. In the defect of active iodide transport (p. 168) and of dehalogenase (p. 169) euthyroidism can also be achieved by giving high doses of iodide (Gilboa, 1963; Vague, 1962). This treatment is of academic interest, but involves the risk of inducing hyperthyroidism, especially in the former syndrome. The routine treatment in these cases is therefore by thyroxine.

F. Thyrotoxicosis

1. Definition, Classification, and Basic Thyroid Hormone Kinetics

When the amount of thyroid hormone available to responsive tissues exceeds physiologic limits, a more or less characteristic clinical picture termed thyrotoxicosis or hyperthyroidism ensues. The serum concentration of triiodothyronine and in most cases also of thyroxine is elevated in hyperthyroidism, except in cases where there is an additional disturbance which lowers the binding proteins of serum, e.g. congenital TBG deficiency or treatment with diphenylhydantoin (p. 143). In such cases it is necessary to measure or estimate the free hormone, which should always be elevated. It is generally agreed that the serum half-life of thyroxine is shortened in hyperthyroidism, while that of triiodothyronine is given as near normal in some studies (McConnon, 1971; Nicoloff,

1972) and shortened in others (WOEBER, 1970). All reports agree that the production rates of both hormones are elevated in hyperthyroidism, except in the rare instances of isolated triiodothyronine-thyrotoxicosis (p. 200). The secretion of nonhormonal iodine, "the iodide leak", is greatly increased in hyperthyroidism (WARTOFSKY, 1971).

The thyroxine-binding capacity of thyroxine-binding prealbumin (TBPA) in serum is decreased by about 40% in patients with hyperthyroidism. In most, though not all, patients thyroxine-binding globulin (TBG) is also lowered (INADA, 1967).

In most cases thyrotoxicosis is due to overproduction of thyroid hormone by the thyroid gland, and very rarely to exogenous hormone administration. The diseases producing thyrotoxicosis are listed in Table 5. The overwhelming majority of cases of thyrotoxicosis are due either to toxic diffuse goiter (Graves' disease) or to toxic nodular goiter. All other causes of hyperthyroidism are rare.

Table 5. Classification of the American Thyroid Association of thyroid disease with thyroid hyperfunction (WERNER, 1969)

1. Toxic diffuse goiter (Graves' or Basedow's disease)
2. Toxic uninodular goiter
3. Toxic multinodular goiter
4. Nodular goiter with hyperthyroidism due to exogenous iodine (Jod-Basedow)
5. Exogenous thyroid hormone excess
6. Tumors
Adenoma of thyroid, follicular
Carcinoma of thyroid, follicular
Thyrotropin-secreting tumor of the pituitary
Hydatidiforme mole with secretion of a thyroid-stimulating substance

In the unselected patient material of the outpatient department of the University of Zurich, three cases of hyperthyroidism were diagnosed among 1000 patients in 1957. In 1964 the incidence was 6 cases per 1000 patients*. In the Department of Medicine, one case of thyrotoxicosis was seen per 100 inpatients. Estimates of the incidence of Graves' disease, the ratio between toxic adenoma and Graves' disease and age and sex distributions are given below.

2. Toxic Diffuse Goiter (Graves' Disease, Basedow's Disease)

The hallmark of this form of hyperthyroidism is a group of almost specific extrathyroidal signs which are not due to thyroid hormone excess and which are absent in other forms of thyrotoxicosis. The most obvious of the changes independent of hormone excess are exophthalmos and periorbital edema, while the other two signs of Basedow's classic Merseburger triad, goiter and tachycardia, are directly related to thyroid overactivity. Other changes independent of thyroid hormone excess are thyroid acropachy, localized myxedema and some other skin changes (see below).

Graves' disease can arise in a normal gland or sometimes in a previously euthyroid multinodular goiter. For obvious reasons the coincidence of Graves' disease and multinodular goiter is more often seen in regions where goiter is endemic (LAMBERG, 1969).

a) Incidence

An extremely careful study on the incidence of Graves' disease in Minnesota has recently been reported from the Mayo Clinic (FURSZYFER, 1970, 1972). The annual incidence per 100000 inhabitants was 19.8 for the entire population and 36.8 for females. From 1935 through 1967 the incidence was remarkably stable, in contrast to the decreasing or increasing incidences in other reports, including reports on epidemics of Graves' disease during world war II in Europe (GREENWALD, 1966). The epidemiologic data on which these reports of epidemics are based are not beyond suspicion (see FURSZYFER, 1972; for review).

All reported series agree that there is a large preponderance of females. In FURSZYFER'S (1972) study the ratio was about 5:1. Graves' disease is rare before the age of 20. Its incidence rises rapidly to reach a peak in the 30-year-old, and then remains almost at a plateau up to the age of about 70. In a very recent study from Denmark, where laboratory screening tests were performed on very broad indications (RONNOV, 1973), the yearly incidence was 14/100000 for the population below the age of 60. For people over 60 the incidence, surprisingly, was seven times higher.

b) Etiology and Pathogenesis

Histological studies suggested early that Graves' disease was characterized by *diffuse* hyperfunction of all the thyroid tissue. This impression was confirmed later when scintiscanning of the thyroid after radioiodine administration showed an increased isotope uptake evenly distributed throughout the tissue. This contrasts with the findings in toxic nodular goiter, where hyperfunction remains localized to parts of the thyroid gland.

* We are grateful to Prof. W. SIEGENTHALER for these data.

Attention therefore naturally became centered on humoral or neural factors as possible causes for the diffuse hyperfunction. An obvious candidate as a humoral factor was pituitary TSH. However, observations that suggested quiescence rather than hyperfunction of the pituitary thyrotrophs, and in particular the occurrence of Graves' disease in hypopituitary patients (FAJANS, 1958; PAZIANOS, 1960; TAUNTON, 1964), provided strong arguments against TSH as a causative factor. More recently, exact measurements by radioimmunoassay have established that serum TSH levels are low in Graves' disease (SCAZZIGA, 1968; PATEL, 1971). This is not to deny that in certain extremely rare cases diffuse toxic goiter may be due to hypersecretion of TSH by a pituitary adenoma (p. 209).

α) Long-Acting Thyroid Stimulator (LATS)

In his search for a humoral factor causing thyroid hyperfunction, ADAMS (1957, 1958, 1965) injected serum of patients with Graves' disease into guinea pigs. He noted that some sera strongly stimulated thyroid activity of the recipient animals and concluded that they contained a pathologic humoral factor. MCKENZIE (1967) soon confirmed this finding by the use of a simpler assay in mice. In his now classic bioassay, mice are first injected with radioiodine, and then after one day to allow for uptake of the isotope into the thyroid gland, the endogenous secretion of TSH is suppressed by the injection of thyroxine, and two days later the test material, usually serum or a concentrate of IgG immunoglobulin, is administered by intraperitoneal injection. Blood is taken just before and at intervals after the injection. The radioactivity in blood, which corresponds to a large extent to the labeled thyroid hormone secreted by the gland, is measured as an index of thyroid function. While the injection of pure TSH produces a rapid rise of the blood radioactivity with a peak at two hours, sera from thyrotoxic patients cause a slow rise with a peak between 8 and 24 hours (MCKENZIE, 1972). The magnitude of the effect is expressed as a precentage of the base-line radioactivity. A rise to 300% of the base-line value 8 hours after the injection is usually considered proof of the presence of thyroid stimulating activity. The technical aspects and the numerous modifications of the MCKENZIE assay have been extensively studied by FLORSHEIM (1970).

Because of the slow onset and prolonged duration of action, MCKENZIE (1967) coined the term Long-Acting Thyroid Stimulator (LATS) for the factor present in the sera of patients with Graves' disease. In the past 15 years most of the research concerned with the etiology and pathogenesis of Graves' disease has been focused on LATS, and an enormous amount of literature has accumulated. The following discussion will touch on only a few aspects of LATS. For more detailed information the reader should refer to one of the reviews available (BURKE, 1968; MCKENZIE, 1967, 1968, 1972; OCHI, 1968; BURKE, 1971).

Fractionation of sera from thyrotoxic patients soon revealed that LATS resided in the γ-globulin fraction and had a sedimentation coefficient of 7S; more detailed analyses confirmed that it was a true IgG immunoglobulin. Both the light an the heavy chain are necessary for biologic activity, but the light chains seem to be less specific as they can be replaced by light chains from other IgG molecules (BROWN, 1968). LATS is produced *in vitro* by lymphocytes from patients with Graves' disease (MCKENZIE, 1967). LATS activity is only partially inhibited by antisera to either λ- or κ-type light chains alone. Both antisera combined will produce 100% inhibition of LATS. This suggests that LATS contains both λ- and κ-type chains and is therefore not produced by a single clone (KRISS, 1968).

LATS duplicates practically every effect of TSH on the thyroid. It produces hyperplasia of the thyroid gland in mice (OCHI, 1969), and stimulates cell mitosis in rat thyroid explants *in vitro* (GARRY, 1970). It stimulates the human thyroid gland when injected into healthy volunteers (ARNAUD, 1965). LATS, like TSH, probably acts via the adenyl cyclase-cyclic AMP system (p. 11) (KANEKO, 1970; BURKE, 1971). LATS stimulates glucose oxidation via the pentose shunt and phosphate incorporation into lipids in a manner indistinguishable from TSH (BURKE, 1971).

Although there exists no doubt that LATS is a true antibody, its corresponding antigen has not yet been clearly identified. Sera of rabbits immunized against human thyroid microsomes contain a thyroid-stimulating activity similar to that of LATS (SOLOMON, 1968 and 1970). LATS can be absorbed onto and reeluted from particulate thyroid cell components which sediment with the microsomal fraction (KRISS, 1964; PINCHERA, 1970). More recently, it has been found that LATS also binds to soluble (nonparticulate) components of thyroid cells (SMITH, 1970; SCHLEUSENER, 1971). These soluble cell components are more abundant in the thyroid on a quantitative basis than the microsomal ones, although they bind less LATS per mg of protein (AMINO, 1971). LATS can be dissociated again from the soluble cell com-

ponents by maneuvers that break up antigen-antibody complexes. SCHLEUSENER (1971) and CHOPRA (1971) have suggested that the soluble cell-sap component which combines with LATS corresponds to solubilized lipoprotein material from the cell membrane. Several such lipoproteins which bind LATS have recently been purified by SATO (1972).

LATS is not always detectable in sera from patients with Graves' disease. In several series of unselected patients with Graves' disease the incidence of LATS varied between 10% and 50% when the serum was tested without first being concentrated. When 15-fold concentrates of IgG are prepared from the patient sera, LATS is found in about 80% of cases (CARNEIRO, 1966). The failure to find LATS in the remaining 20% of patients is probably best explained by the insensitivity of the mouse bioassay. Indeed, ADAMS (1971) presented good evidence that LATS-negative sera from patients with Graves' disease all contain an IgG immunoglobulin which reacts with human but not with murine thyroid extracts. He suggested that this globulin, named *LATS protector*, stimulated the human thyroid gland, but did not react with the mouse thyroid gland and was thus not detectable in the ordinary bioassay (SHISHIBA, 1973).

Patients with pretibial myxedema (p. 187) usually have high titers of LATS (KRISS, 1964), although this is not an undisputed point (SCHERMER, 1970). The initial association of severe ophthalmopathy with high LATS titers has not been confirmed by later studies (p. 201). There is no very close correlation between the incidence of LATS and the severity of thyrotoxicosis, but patients with all three cardinal manifestations (thyroidal, cutaneous, ocular) have a higher incidence than patients with only one feature (LIPMAN, 1967). After treatment with antithyroid drugs (PINCHERA, 1969) or after thyroidectomy (see p. 194) the concentration of LATS tends to decrease, while after radioiodine therapy it rises temporarily (PINCHERA, 1969).

At first glance the presence of LATS offers an obvious explanation for the pathogenesis of thyroid hyperfunction in Graves' disease. Surprisingly, in recent years several authors have expressed well-founded doubts about the concept that LATS causes the thyroid hyperfunction of Graves' disease. Eloquent reviews have been written both supporting (HALL, 1970; MCKENZIE, 1972) and rejecting (CHOPRA, 1970) a pathogenic role for LATS. Neonatal hyperthyroidism is an experiment of nature which provides strong evidence in support of the involvement of LATS. It is a very rare disease and occurs only in babies of mothers who have very high LATS titers. LATS is present in the serum of the newborn, and the titer declines spontaneously in parallel with disappearance of hyperthyroidism (MCKENZIE, 1964; SUNSHINE, 1965). The argument that LATS remains undetectable in 20% of patients despite the use of IgG concentrates is easily countered by pointing to the insensitivity of even the refined bioassays. However, the strongest argument against a causative role of LATS is the fact that it often remains detectable in serum when patients go into remission or when the thyroid gland becomes normally suppressible by triiodothyronine again (see p. 191 for suppression test) (CHOPRA, 1970; SELLERS, 1970; SIVERSTEIN, 1970). The dispute over the role of LATS in Graves' disease has not yet been satisfactorily settled.

It must be mentioned that LATS also occurs in patients with euthyroid ophthalmic Graves' disease (p. 202) and in cases of idiopathic hypothyroidism (LIDDLE, 1965). The spontaneous progression of Graves' disease into idiopathic hypothyroidism has been observed in few cases (LEVITT, 1954) and was described by old clinicians as "burning out" of thyrotoxicosis. LATS has occasionally been found in euthyroid relatives of patients with Graves' disease (WALL, 1969).

β) Other Immunologic Abnormalities in Graves' Disease

It has long been known that the thymus is enlarged in Graves' disease, and recent publications have re-emphasized the presence of cervical lymph node enlargement (MAHAUX, 1969). Classic antibodies to either thyroglobulin or thyroidal microsomal antigen can be demonstrated by conventional techniques in about 60% of patients with Graves' disease. The titers are rarely as high as in chronic lymphocytic (Hashimoto's) thyroiditis. With the aid of a very sensitive competitive binding radioassay, MORI (1971) found antimicrosomal antibodies in 98% and antithyroglobulin antibodies in 89% of patients with Graves' disease, while in control subjects the incidence of both antibodies was only about 20%. In chronic lymphocytic thyroiditis, MORI (1971) found an identical incidence of antibodies. This supports the view that the two conditions are etiologically related, or in other words, varying manifestations of the same underlying disease process. Several pairs of monozygous twins have been reported, one of whom suffered from Graves' disease and the other from chronic lymphocytic thyroiditis (JAYSON, 1967;

DONIACH, 1967; CHERTOW, 1973). Both diseases can coexist in one individual (FATOURECHI, 1971).

WERNER (1972) found by immunofluorescence that IgG, IgM and IgE as well as complement was deposited along the basement membranes of diffuse toxic goiters. Recently, VOLPE (1972) and LAMKI (1973) provided evidence of delayed cell-mediated autoimmunity to thyroid antigens in patients with Graves' disease.

Patients with Graves' disease have a relatively high incidence of antibodies to parietal gastric cells and intrinsic factor (DONIACH, 1963), and they suffer from pernicious anemia about 5 times more frequently than controls (p. 188).

Cutaneous reactivity to tuberculin and to dinitrochlorobenzene appears to be absent in most patients with Graves' disease, which points to a defect in delayed-type hypersensitivity (BRODY, 1972).

In summary, a number of immunological abnormalities are present in the sera of most patients with Graves' disease, viz. classic complement-fixing antibodies to well defined antigens, a peculiar antibody which acts as a thyroid stimulator, and finally disturbances of delayed hypersensitivity. The true antibody nature of LATS has long been doubted by immunologists, but the present evidence leaves little doubt on this point. All these facts suggest that Graves' disease is a disorder of autoimmunity in which immune tolerance towards the thyroid and other tissues breaks down.

γ) Familial Incidence

Familial incidence of Graves' disease has been well documented (BARTELS, 1941; MARTIN, 1951), but the exact mode of genetic transmission has not been established. It appears that the liability to develop thyroid antibodies is inherited by polygenic control (Editorial Brit med. J. 1973). About one quarter to one fifth of patients have near relatives also suffering from the disease. As outlined above, Graves' disease and chronic lymphocytic thyroiditis may occur in the same family. HASSAN (1966) has reported 4 pairs of identical twins who suffered from Graves' disease. In each pair of twins, both had identical autoantibodies in the serum. SKILLERN (1972) has recently written a concise review on the genetics of Graves' disease.

δ) Triggering External Events

Graves' disease may follow sudden or prolonged psychological stress. Many authors have therefore emphasized the importance of the central nervous system in the etiology of the disease. Severe fright induced in wild rabbits has produced tachycardia, hyperthermia, exophthalmos and stupor. This so called "Schreck-Basedow" rapidly leads to death (EICHHOFF, 1949). However, the presence of true hyperthyroidism in this syndrome has never been well documented; the protein-bound iodine has been found to be normal (SCHÄFER, 1965; OBERDISSE, 1967).

Depending on the authors, psychological trauma is incriminated as a cause of Graves' disease in 4% to 97% of cases. No carefully controlled studies have been reported, however, and the issue remains unsettled. The influence of the nervous system on the thyroid gland has been reviewed by MCKENZIE (1967).

Weight-reducing diets, especially when combined with the administration of thyroid hormone, have been accused of triggering Graves' disease, but again controlled studies are not available. In so-called "Jod-Basedow" ingestion of large amounts of iodine produce hyperthyroidism. Interest in this syndrome has recently been revived (p. 209), but it should be remembered that "Jod-Basedow" is not true Graves' disease, but nodular goiter rendered toxic by an excessive iodine supply.

c) Pathologic Anatomy of the Thyroid and Pituitary Glands

In most cases of Graves' disease the thyroid is enlarged. As a rule the hyperplasia is diffuse, but when Graves' disease is superimposed on pre-existant euthyroid nodular goiter the gland may be multinodular (so-called "struma basedowificata") and the distinction from toxic nodular goiter on pathologic grounds alone may be difficult. The vascularity of the thyroid gland is increased. The follicles vary widely in size and shape, and contain little colloid. The epithelial cells increase in depth and form papillary structures. Lymphoplasmocytic infiltrates with germination centers are seen in the stroma (Fig. 15). Some epithelial cells become enlarged and contain granular, slightly acidophilic cytoplasm and a bizarre nucleus (so-called oncocytes, oxyphils, or Hürthle cells). OLEN and KLINCK (1966) found a surprisingly high incidence (2.5%) of thyroid carcinoma, sometimes of only microscopic size, in a series of toxic diffuse goiters. Such carcinomas should therefore always be looked for carefully in surgical specimens.

Changes in the anterior pituitary gland are not very striking. Degenerative changes of the chromophilic cells have sometimes been observed.

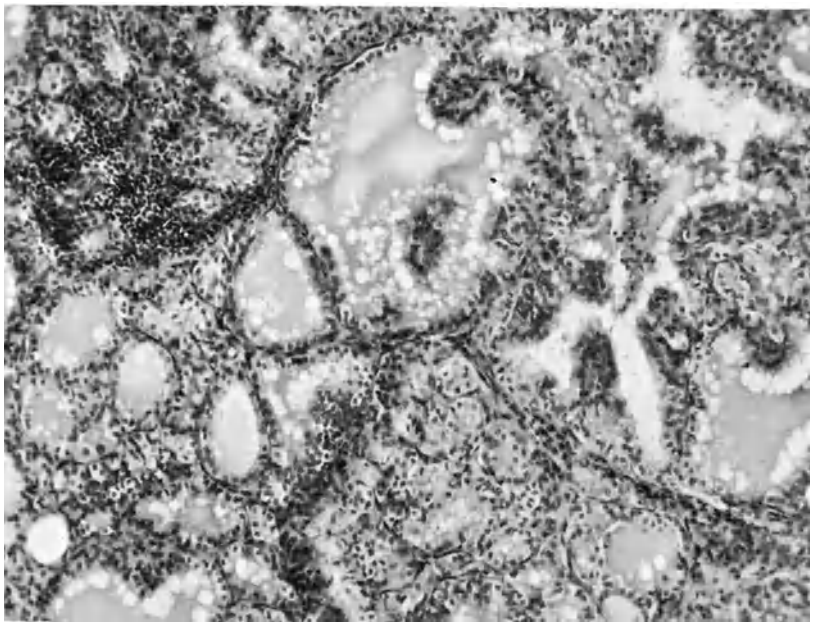


Fig. 15. Histologic picture of a goiter from Graves' disease. Note the wide variation in follicle size, the diminished colloid content, the infoldings of the epithelium into the lumen and the elongated epithelial cells. A lymphocytic infiltrate is seen at upper left (Hematoxilin-Eosin, × 140)

d) Clinical Features

α) History and General Clinical Findings

In some cases, Graves' disease develops insidiously over some months or years, but more often it has a sudden onset within a few days. The symptoms are often quite nonspecific, and are sometimes difficult to differentiate from those of patients with "functional" emotional disturbances. As a rule, patients with functional emotional conditions complain of severe subjective distress with few objective findings, while in patients with Graves' disease the changes are often first noted by the relatives before the patient mentions any symptoms. Nervousness, fatigue and insomnia are frequently reported, but they are of course nonspecific. On questioning the patient admits to increased perspiration. Warm intolerance may become apparent in the use of less blankets at night and less clothes during the day. Palpitations and sometimes exertional dyspnea may be prominent. Some patients complain of hair loss. Increased appetite combined with loss of weight is very suggestive of hyperthyroidism.

Emotional and mental changes are almost always present. There is generalized irritability and emotional tension. The patient may be hyperactive, sometimes euphoric, but easily fatigued. He complains of difficulty in concentrating. Some patients are frankly hyperkinetic and cannot sit still for a moment. The sexual drive sometimes increases initially and then decreases. The patient himself is often aware of his mental changes. In extreme forms patients become frankly manic, or psychotic,

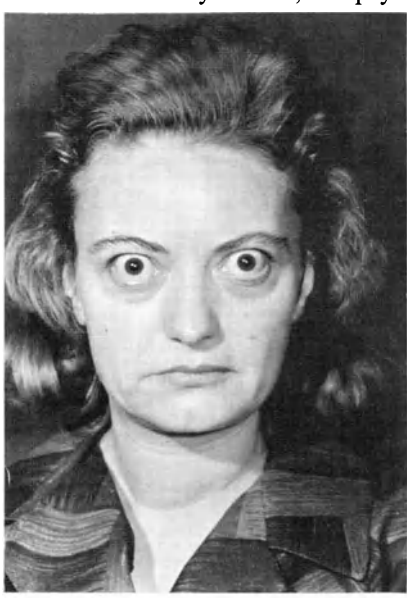


Fig. 16. 37-year-old woman with Graves' disease of 7 years duration and class 1 ophthalmopathy. The patient had developed pretibial myxedema during a first course of drug treatment. Three years later a subtotal thyroidectomy was performed because of a recurrence of hyperthyroidism. With the improvement of hyperthyroidism the pretibial myxedema worsened and the patient developed proptosis, clubbing of fingers and swelling of interphalangeal joints (acropachy). Radioiodine treatment finally provided complete control of hyperthyroidism, but the ophthalmopathy and the pretibial myxedema remained unchanged

or delirious. Sometimes the disease is indistinguishable from acute schizophrenia. Patients occasionally become hypoactive and depressed (apathetic thyrotoxicosis; THOMAS, 1970; see p. 199).

On physical examination the patient strikes the examiner by his tense facial expression (Fig. 16). He looks scared. He enters the room with hyperkinetic, badly coordinated movements and answers questions quickly and overzealously. During ward rounds he may reveal the nature of his illness by shooting up in bed and sitting up as soon as the doctor approaches.

β) Thyroid Gland

Goiter is almost always present, although in some cases the thyroid gland remains surprisingly small. The enlarged gland is usually clearly visible, since most patients are thin. Palpation shows it to be diffusely enlarged, both lateral lobes, the isthmus, and sometimes the pyramidal lobe being affected. It is usually two to four times as large as normal (40 to 80 g), but in young patients the goiter may become much bigger. When Graves' disease is superimposed on a previously euthyroid goiter (LAMBERG, 1969, p. 260), the gland may be multinodular and the clinical differentiation from toxic multinodular goiter may be quite difficult unless clear-cut ocular and dermal changes are present.

The goiter of Graves' disease is of characteristic fleshy or rubbery firmness. It rarely causes local pressure symptoms or pain. The increased vascularity produces a systolic bruit and a continous humming murmur in about two-thirds of cases, while in euthyroid goiter this is a rare finding.

γ) Ocular Changes (cf. p. 201)

Protusion of the bulbi (exophthalmos) is found in about one quarter of all cases. Very often the exophthalmos is unilateral or is more pronounced on one side. For objective assessment the exophthalmos should be measured with a special instrument, e.g. the Hertel exophthalmometer, in every patient. Values of more than 20 mm (corneal apex to lateral orbital rim) represent probable exophthalmos, and values of more than 23 mm definite exophthalmos. The eyelids may be swollen, puffy and sometimes grossly edematous (periorbital myxedema, Fig. 18). In severe cases, chemosis and corneal ulcers may be present. Few diseases need be considered in the differential diagnosis. Protrusion of the bulbi may be due to a genetically transmitted trait. Sometimes it is due to a retrobulbar neoplasm, and rarely to accumulation of lipids, as in Hand-Schüller-Christian disease. When a retrobulbar tumor is present, the resilience of the bulbi is often diminished, while in endocrine ophthalmopathy the bulbs may be displaced backward by gentle pressure.

Lid retraction produces the characteristic stare (Fig. 16), a sign which is independent of the presence of exophthalmos. When the patient is instructed to follow the examiner's finger as

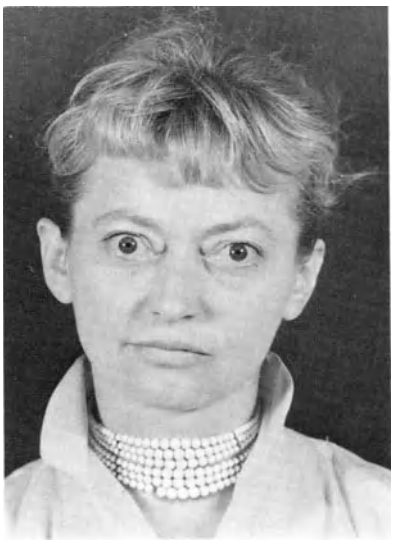


Fig. 17. 37-year-old patient with Graves' disease and class 3 ophthalmopathy. One year after subtotal thyroidectomy there was worsening of the ocular signs. Note the edema of the upper lid with protrusion of the lis nasally

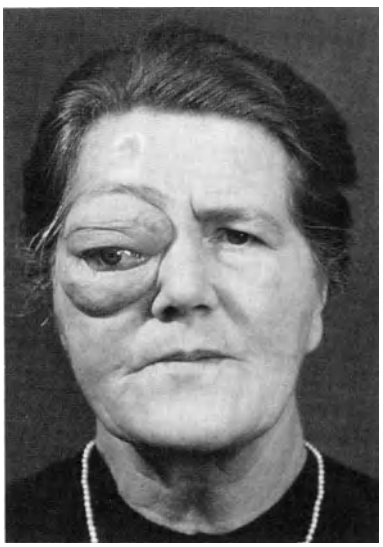


Fig. 18. 66-year-old euthyroid patient with class 6 endocrine ophthalmopathy of 16 years' duration. Surgical decompression provided only temporary relief. The patient was blind in the right eye. (Courtesy of Prof. WITTMER, Kantonsspital Zürich)

it is moved downwards in front of him, the typical lid lag becomes apparent, i.e. a narrow white rim of the sclera becomes visible between the upper lid and the cornea (Graefe's sign).

Due to infiltrative changes of extra-ocular muscles, disturbances of eye movement appear in severe cases (Fig. 19). The earliest sign may be weakness of convergence and consequent diplopia on viewing near objects (Moebius' sign).

The eye changes, in particular exophthalmos, periorbital edema, and muscle involvement, take a course which is quite independent of that of the thyroid hyperfunction. In extreme cases they may ultimately develop into malignant exophthalmos. The pathogenesis, clinical course and treatment of endocrine ophthalmopathy are discussed in detail on p. 201.

δ) Cardiovascular System

Cardiovascular manifestations are prominent in thyrotoxicosis, and untreated patients suffer from and often die of heart disease.

The biochemical relationships between thyroid hormone and the heart have been outlined in detail on p. 146. Graves' disease is a classic example of a high-cardiac-output state. The cardiac output is significantly more raised than one would expect on the basis of the increased oxygen consumption of the peripheral tissues (DEGROOT, 1970). GRAETTINGER (1959) has analyzed the hemodynamics of patients with long-standing hyperthyroidism, both with and without heart failure. The cardiac output of seven patients in failure was normal in four and elevated in three.

Tachycardia is an almost obligatory sign of thyrotoxicosis. Characteristically the pulse rate remains high during sleep. Sometimes premature beats or paroxysmal supraventricular tachycardia are present. The pulse rate is usually between 100 and 120, but in severe cases it may reach 160. β-Adrenergic blockers and guanethidine reduce the pulse rate in hyperthyroidism (HOWITT, 1967; GROSSMAN, 1971), but as outlined on p. 146, this is no proof that thyroid hormone acts via catecholamines.

The diastolic blood pressure is low and the systolic blood pressure may rise, especially in older patients. The wide pulse pressure produces systolic sounds over the great arteries. The hyperdynamic cardiovascular state causes bothersome palpitations to the patients, particularly after physical exercise. The apical cardiac impulse is forceful and sometimes displaced to the left, which is often mistaken for a sign of left ventricular hypertrophy. Cardiac sounds are loud, and a short systolic murmur (systolic scratch) is heard along the left sternal border. Diastolic murmurs are rare. Auricular fibrillation is frequent in older patients. Exertional dyspnea and retrosternal pressure are frequent symptoms, but true angina pectoris is rare.

The study by SANDLER (1959) provides convincing evidence that thyrotoxicosis may damage an otherwise normal heart. Of his 462 thyrotoxic patients treated with ^{131}I, 150 had heart disease. Of these, 86 had associated hypertensive, coronary or valvular heart disease. In 64 patients there was no "organic" heart disease, and the cardiac symptoms were attributed to thyrotoxicosis alone. Auricular fibril-

a b c

Fig. 19. a) 28-year-old man with hyperthyroidism due to Graves' disease. b) Iatrogenic hypothyroidism 6 months after subtotal thyroidectomy. c) 6 years later; proptosis and paresis of the m. rectus superior on the right side. The patient had taken his thyroid hormone replacement inconsistently. Class 4 ophthalmopathy

lation and congestive heart failure were very frequent in this group.

It is important to realize that the response of the heart to digitalis glycosides is altered in hyperthyroidism (BUCCINO, 1967). It is a familiar clinical observation that in hyperthyroidism with auricular fibrillation the ventricular rate has a poor response to conventional doses of digitalis. This has been borne out by careful studies in man and dog. FRYE (1961) compared the response to digoxin in euthyroid patients with auricular fibrillation before and after administration of 225 μg triiodothyronine per day. The dose of digoxin necessary to maintain a ventricular rate of 70 was approximately 0.2 mg/day in the euthyroid and 0.8 mg/day in the hyperthyroid state. Interestingly, the higher dose was tolerated without side effects. Clinically, his subjects exhibited only mild signs of thyrotoxicosis while under triiodothyronine. It may therefore be anticipated that in severe hyperthyroidism the digoxin requirement may be even higher. According to MORROW (1963) the digitalis requirement of the thyrotoxic dog heart is increased only if the atrioventricular refractory period is measured as an index, while the contractile force responds normally to conventional digitalis doses. The dose of ouabain causing ventricular arrhythmia was about 50% higher in dogs rendered hyperthyroid (MORROW, 1963).

The electrocardiogram shows the afore-mentioned arrhythmias and also some nonspecific changes in the ST-segment. In about 50% of cases autopsy reveals myocardial changes such as hypertrophy, edema, localized necrosis, fibrosis and sometimes lymphocytic infiltrates.

ε) Respiratory System

Hyperthyroid patients often complain of dyspnea on exertion, even when there is no heart failure or pulmonary disease. Pulmonary function studies have revealed decreased compliance and increased functional residual capacity but otherwise few significant changes. The best explanation available for the dyspnea is that it is caused by weakness of the respiratory muscles due to thyrotoxic myopathy (STEIN, 1961; MASSEY, 1967). These changes are reversible after treatment.

ζ) Neuromuscular System

Hyperthyroidism has profound effects on the central and autonomous nervous system, causing increased irritability, nervousness, insomnia, tremor, increased perspiration and many other signs or symptoms which are often also present in patients with simple "functional" complaints or emotional instability.

The fine tremor, one of the cardinal signs, varies widely in intensity. Its frequency is about 8–10/sec. Sometimes it will only be revealed by palpation of the out-stretched hands. Tendon reflexes are brisk and relaxation is quick. This sign may be quantitatively evaluated by recording the ankle jerk (p. 241).

In its extreme form, thyrotoxicosis leads to generalized encephalopathy (p. 198). Cerebellar dysfunction has sometimes been reported. It is reversible after treatment. Polyradiculitis, optic neuritis and Parkinsonism do not occur with a higher frequency than in a control population. Non-specific electroencephalographic changes are often present. They may persist even when the hypothyroidism is properly treated (SIERSBAEK, 1972).

Muscular weakness is prevalent in hyperthyroidism, but it is often only revealed by careful examination. On specific questioning the patient admits that he has to pull himself with his hands when climbing stairs. The muscular signs of hyperthyroidism resemble those of myasthenia gravis, while hypothyroidism produces a picture more reminiscent of myotonia. The clinical changes are difficult to explain on a biochemical or electrophysiologic level (HAVARD, 1963), but peripheral neuropathy (LUDIN, 1969) and a loss of motor units per motor neuron (MCCOMAS, 1973) have been implicated.

NOSEDA (1967) found signs of chronic hyperthyroid myopathy in about 20% of cases with Graves' disease and in 10% of patients with toxic nodular goiter, while RAMSAY (1966, 1968) detected evidence of muscular dysfunction in almost 100% of cases.

In *chronic thyrotoxic myopathy*, fatigue, muscular weakness sometimes to the extent of paresis, and muscular atrophy are prominent and may be the presenting symptoms of Graves' disease. The muscles of the shoulder and pelvic girdles and the small hand muscle are the ones predominantly affected. A typical sign is the "signe du tabouret" recorded by French clinicians; the patient is unable to stand up from a low chair without using his hands. The onset of myopathy can be rapid, within weeks, or insidious, extending over years. The severity bears little relation to the degree of thyroid hyperfunction, but it is usually reversible after treatment of the latter. The exact biochemical basis of the muscular dysfunction is not known. Thyrotoxic patients excrete elevated amounts of creatine in urine. A greater fraction of an exogenous creatine load is excreted in the urine than in normal subjects. These changes may be related to disturbances in mitochondrial res-

piration (p. 145), but careful examination of muscle mitochondria from patients with thyrotoxic myopathy have yielded no positive evidence supporting this assumption (STOCKER, 1968).

Pathological examination shows the muscles to be edematous. The interstitial space is filled with fat cells and lymphocytic infiltrates. The muscle fibers are atrophic, the nuclei swollen. Transverse sections show semilunar intracellular inclusions of mucopolysaccharides.

Acute thyrotoxic myopathy occurs superimposed on thyrotoxic crisis and presents with a picture of bulbar paralysis with impaired swallowing and speech.

Myasthenia gravis may coexist with thyrotoxicosis and the association is more frequent than can be attributed to chance (ENGEL 1972). When present together, the two diseases progress independently. Patients with myasthenia gravis develop hyperthyroidism quite frequently, while hyperthyroid patients become myasthenic quite rarely, but still more frequently than can be attributed to chance. It is interesting to note that the thymus may be enlarged in both diseases.

Thyrotoxic periodic muscle paralysis is a very rare complication of hyperthyroidism. It seems to be observed most frequently in the Far East (OHINAKA, 1957; OKIHIRO, 1965; SHIZUME, 1966; MCFADZEAN, 1967; ENGEL, 1972). The symptoms are identical to those of periodic hypokalemic paralysis, and signs may appear in rest periods after muscular exertion, after psychologic stress, or during exposure to cold. Weakness of the lower extremities is prominent. The episodes last about 12 hours. They are not always accompanied by hypokalemia, but arteriovenous differences of the potassium concentration have been found (SHIZUME, 1966). After treatment of the hyperthyroidism, the attacks of paralysis abate. RESNICK (1969) studied one patient who had an elevated aldosterone secretion rate in addition to the classic findings.

In endocrine ophthalmopathy, dysfunction of the extra-ocular muscles may be prominent. This disorder is discussed in detail on p. 201.

η) Bones and Joints; Calcium Metabolism

Osteoporosis is a frequent finding, especially in older patients. MEGLIOLI (1966) found radiologic evidence of osteoporosis in about 7% of cases. FRASER (1971) found that decreased radiologic bone density was particularly frequent in thyrotoxic women over 50. In younger patients, treatment of thyrotoxicosis improved the bone demineralization, but in the older patients the changes persisted and fractures were seen.

The pathologic anatomical changes in the bones are diverse. Fibro-osteoclastic foci eroding the cortical spongial bone are frequent. Signs of osteomalacia with osteoid brims are occasionally seen. Classic osteoporosis sometimes also occurs.

Calcium and phosphorus excretion in stool and urine is increased (KRANE, 1956; HARDEN, 1964). Serum calcium and phosphate are usually within normal limits, but moderate hypercalcemia is by no means rare (KOENIG, 1959; BAXTER, 1966). Severe hypercalcemia which responds well to corticosteroid administration is rare (SATALINE, 1962; HARPER, 1970). Many theories have been proposed to explain the hypercalcemia of Graves' disease, but none is entirely satisfactory (PARFITT, 1970). Calcium balance is usually negative and it has been claimed that the administration of 2 g of calcium per day will reverse this.

After thyroidectomy serum calcium often falls to hypocalcemic levels. An obvious explanation is that the parathyroid glands have been inadvertantly removed or severed. However, MICHIE (1971) has shown that a more frequent cause of transient post-thyroidectomy hypocalcemia is increased calcium uptake by the calcium-depleted bones.

Thyroid acropachy is a very rare complication of Graves' disease. In many ways it resembles hypertrophic osteoarthropathy of chronic pulmonary disease, and it presents with swelling and clubbing of the distal phalanges. There is cuff-like hypertrophy of subperiostal bone, especially in the phalanges and the distal ends of the bones of the arms and legs. Thyroid acropachy is often associated with pretibial myxedema and exophthalmos. The lesions may be tender. There is no effective treatment for the disorder.

ϑ) Skin

In its function as thermoregulatory organ the skin has to dissipate the extra heat produced by hyperthyroidism, a process which explains many of the dermal signs. The skin feels warm and moist. Perspiration may become excessive even after moderate exercise. The signs are most conspicuous in the palms. Warm moist hands are very suggestive of hyperthyroidism, but are frequently also found in patients with febrile infections. Cold hands virtually exclude the diagnosis of hyperthyroidism. The face is usually pale, but intermittent flushing sometimes occurs. Sometimes patchy erythema of the face and trunk is seen. Dermographia is often present sometimes leading to urticaria and itching. The skin is thin and soft. Hyperpigmen-

tation similar to that seen in Addison's disease is sometimes a prominent feature. KIRKEBY (1963) has attributed this sign to an increased ACTH level allegedly necessary to maintain the high cortisol secretory rate in hyperthyroidism. Some patients suffer from widespread vitiligo (OCHI, 1969).

The scalp hair is thin and brittle. Hair loss, sometimes progressing to alopecia is a frequent sign. There is often premature graying of the hair.

Some patients suffer from separation of the distal end of the nails from the underlying bed (onycholysis, Plummer's nails).

Localized pretibial myxedema (Fig. 20) is a peculiar dermal change which is histologically identical to the skin changes of hypothyroidism. The deeper dermis contains swollen fibers and is infiltrated by mucilagenous material, mostly hyaluronic acid (SISSON, 1968). It is seen in 3–4% of patients with Graves' disease and is often associated with ophthalmopathy. Its relation to LATS has been discussed on p. 180.

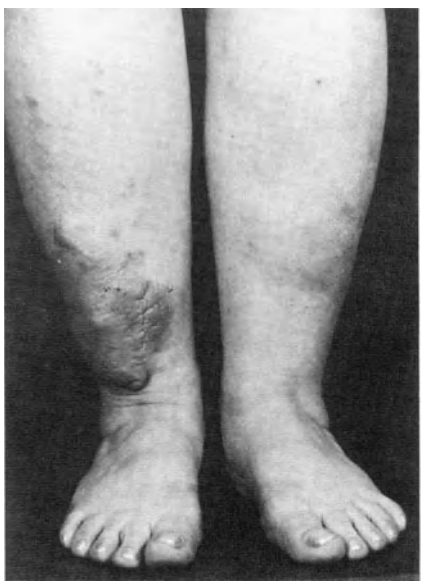


Fig. 20. Pretibial myxedema in the same patient as shown in Fig. 16. The lesion is much more severe on the right side. The case history is summarized in Fig. 16

Localized pretibial myxedema presents as plaques 5–20 cm in diameter over the lateral or anterior aspects of the legs. Sometimes they extend to the dorsal aspect of the feet. The fact that the hair follicles are retracted makes the lesions look like pigskin. No effective treatment is available. Sometimes the lesions clear spontaneously. Systemic corticosteroids will produce regression, but only for as long as the medication is given. Good results have been claimed by KRISS (1967) with topical steroid application.

ι) Gastrointestinal Tract and Liver

The frequency of bowel movements is increased in most patients with Graves' disease (BAKER, 1971), and about one third have diarrhea. The cause is increased peristalsis of the intestines.

If diarrhea is severe, steatorrhea may be present. It disappears after treatment of the hyperthyroidism (CRANE, 1966). Steatorrhea is due to agreatly increased fat intake and not to malabsorption (THOMAS, 1973). Appetite is increased and some patients really crave for food. In rare cases, however, patients are anorectic. Achlorhydria refractory to histamine and serum antibodies to gastric parietal cells are found in about one third of patients. Antibodies to gastric parietal cells and intrinsic factor are present less frequently, and frank pernicious anemia occurs in about 2% of cases (p. 188).

Liver function is only mildly impaired in most cases of thyrotoxicosis. In exceptional cases jaundice with unconjugated hyperbilirubinemia develops. GREENBERG (1964) has attributed this to a concomitant disturbance of bilirubin transport or conjugation which becomes manifest with the increased erythrocyte turnover in thyrotoxicosis. Serum transaminases are often slightly elevated and bromsulfthalein retention is pathologic in about half of the cases (WEBER, 1968). Alkaline phosphatase is also often elevated, but this may be due to bone disease.

In patients who die of thyrotoxicosis, severe liver changes with centrolobular necrosis or even massive hepatic necrosis are found. Some of the changes resemble those of circulatory failure, and one theory postulates that decreased liver perfusion inadequate to meet the increased metabolic needs is responsible for the liver pathology. Many of the changes must be attributed to terminal events indirectly related to thyrotoxicosis, and percutaneous liver biopsy in moderately ill patients has revealed a far less impressive picture. KLION (1971) found only mild nonspecific changes, such as infiltration of the portal tracts by lymphocytes and moderate steatosis. Electron microscopy showed enlarged mitochondria.

κ) Lipid and Carbohydrate Metabolism

The effects of thyroid hormone on lipid metabolism have been briefly reviewed on p. 147.

Serum cholesterol is often lowered in hyperthyroidism. NIKKILÄ (1972) found elevated plasma triglycerides, which he attributed to

increased synthesis. Post-heparin lipolytic activity was high in his studies. Interestingly, some of the triglyceride abnormalities did not return to normal after treatment of hyperthyroidism.

The effects of thyroid hormone on glucose metabolism are complex and not fully understood. On one hand thyroid hormone appears to enhance peripheral glucose consumption, on the other it stimulates gluconeogenesis (p. 148). Glucosuria is found in about 38% of thyrotoxic patients, a much higher incidence than in controls. However, only 2 to 3.3% of thyrotoxic patients suffer from overt diabetes mellitus, an incidence similar to that in the population as a whole (KOSAK, 1971).

λ) Blood and Lymphatic System

Mild anemia not responsive to the administration of vitamin B_{12}, folate or iron is a frequent finding in hyperthyroidism. The pathogenesis is not known. Compared to thyrotoxic patients with normal hemoglobin, the anemic patients have a decreased incorporation of iron into red cells (RIVLIN, 1969).

Achlorhydia and antibodies to gastric parietal cells and intrinsic factor are often found in Graves' disease (WILLIAMS, 1966: ARDEMAN, 1966; WANGEL, 1966), and frank pernicious anemia occurs in about 2% of cases, about 5 times as frequently as in a matched control population (SCHILLER, 1968, FURSZYFER, 1971).

Lymphocytosis is often present in the peripheral blood and the cervical lymph nodes may be enlarged (MAHAUX, 1971). In some cases the enlarged nodes may progress into true lymphoma (ULTMANN, 1963). The spleen is palpable in one third of cases and the thymus is often enlarged (MICHIE, 1967).

Warfarin-induced hypoprothrombinemia is enhanced by thyrotoxicosis (VAGENAKIS, 1972). Hypoproteinemia and edema have been described (PINCHERLE, 1965).

μ) Kidney

Moderate polyuria is probably caused by increased glomerular filtration due to renal hypercirculation. The response to vasopressin is normal, but there is slight impairment of the mechanism of urine concentration, possibly because the sodium concentration in the medulla is diminished (CUTLER, 1967). The maximal tubular reabsorption of glucose is enhanced. If hypercalcemia is present, reversible impairment of kidney function and sometimes renal acidosis may occur (LABHART, 1968).

ν) Other Endocrine Glands

Men with Graves' disease sometimes suffer from gynecomastia (LARSON, 1963; ASHKAR, 1970), which is reversible after treatment of the hyperthyroidism. The cause of gynecomastia appears to be estradiol-17β, which is moderately elevated in most male patients and markedly so in those with gynecomastia (CHOPRA, 1972).

Of patients with Addison's disease 3–4% also suffer from Graves' disease, a coincidence which is about 10 times higher than is attributable to chance (KAPPELER, 1965; GASTINEAU, 1965; BURKE, 1965).

Cortisol turnover is greatly increased in thyrotoxicoses and its half-life is shortened. Bursts of cortisol secretion are greatly increased in frequency in thyrotoxic patients (GALLAGHER, 1972).

Hyperthyroidism in pregnancy is discussed on p. 199.

ξ) Natural Course of Graves' Disease

In some cases Graves' disease has an insidious onset, but often the patient reports a sudden onset, often related to emotional stress. Owing to the fact that almost every patient in whom the disease has been diagnosed receives one form of treatment or another, few studies on the natural course are available. The early clinicians knew that Graves' disease took an unpredictable course with periods of continuous mild hyperthyroidism, acute exacerbations, complete remission and severe relapse. In about 10% of patients the disease progressed relentlessly and ended in death. In men the prognosis was considered to be much more serious than in women. MURRAY (1903) followed 40 cases regularly over a period of 11 years; 7 patients died of the disease, 2 remained stationary, 14 were improved to some extent, 8 were greatly improved and 9 recovered fully. Of the latter group, 2 patients had a relapse later. The relatively benign course of thyrotoxicosis has recently been confirmed by a small study (MCLARTY, 1971).

In rare cases Graves' disease develops into spontaneous hypothyroidism, probably due to superimposed atrophic thyroiditis.

σ) Differential Diagnosis and Diagnostic Value of Clinical Features (Diagnostic Indices)

Many patients referred to thyroid centers for suspected hyperthyroidism turn out to be euthyroid, and the most frequent diagnosis in these cases is neurotic personality disorder or anxiety state. Tachycardia, palpitation, tremor,

hyperhidrosis, dermographia are common in both psychiatric and thyrotoxic patients. A good distinctive clinical sign is that in the thyrotoxic the moist hands are always warm, while in the neurotic they are cool.

Chronic infections, neoplasia and pheochromocytoma are often associated with hypermetabolism and weight loss, and may therefore be mistaken for hyperthyroidism. A typical lid lag which is often present in these states may add to the confusion. Severe diabetes mellitus typically presents as weight loss with increased appetite and must of course be ruled out in cases of suspected thyrotoxicosis.

A high cardiac output or a hyperkinetic heart are also observed in pheochromocytoma, Paget's disease, fibrous dysplasia of bone (FISCHER, 1970), arteriovenous fistulas, beriberi heart disease and sometimes in subvalvular aortic stenosis (GORLIN, 1962; SLOMAN, 1967).

Lead intoxication with weight loss, tremor, mental changes and diarrhea may occasionally be mistaken for thyrotoxicosis. Intoxication with dinitrophenol typically leads to hypermetabolism. A single very interesting case of idiopathic hypermetabolism attributed to a defect of respiratory control in muscle mitochondria has been described by LUFT (1962). Weight loss, muscular weakness and hyperpigmentation of the skin are common to both Addison's disease and Graves' disease.

The experienced doctor will have no trouble in differentiating the above states from true Graves' disease by clinical observation and will therefore spare the patient additional expensive laboratory tests in most cases.

The signs and symptoms discussed above do not all carry the same weight in differentiating euthyroid from hyperthyroid patients. CROOKS (1959) has developed a very useful diagnostic index based on discriminant function analysis, which allows confirmation or exclusion of hyperthyroidism on clinical grounds alone in most cases. This index is particularly useful for doctors who are not very familiar with thyroid disease; a certain numerical value or weighting is assigned to each sign or symptom, according to its discriminant significance. GURNEY (1970) has extended this index to include some psychiatric items, which allows clearer separation from neurotic disorders and anxiety states. It is striking that both these diagnostic indices omit some textbook symptoms or signs of Graves' disease, e.g. diarrhea or hair loss. Obviously, these features do not have enough discriminant function. It is a common observation that the clinical diagnosis of hyperthyroidism is more difficult in older patients (HARVEY, 1971). In the elderly, thyrotoxicosis rarely presents with all the classic manifestations, and oligo- or monosymptomatic forms are often seen, e.g. forms with predominantly cardiac or predominantly mental signs. GURNEY (1970) has taken account of this fact by incorporating an age-correction factor into his diagnostic index.

π) Laboratory Tests

It is customary, although not strictly necessary, to confirm the clinical diagnosis of Graves' disease by at least one or two laboratory tests. In clinically doubtful cases several tests are usually carried out.

Not all tests carry the same weight for the distinction between euthyroidism and hyperthyroidism. The discriminant functions of different tests have been compared in several studies (see p. 244). Most authors have found that the "best" single tests are the radioiodine uptake within a short interval (2 to 4 hours) and tests measuring thyroid hormone concentration (serum total thyroxine in combination with the T_3 resin uptake). The basal metabolic rate obtained an intermediate rating and the serum cholesterol a rather low one in most of the above studies.

In patients for whom the usual laboratory tests give equivocal results it is customary to carry out a triiodothyronine suppression test (p. 242). This test will distinguish between a high radioiodine uptake due to dietary iodine deficiency and one due to Graves' disease.

In rare patients the diagnosis is established only by a therapeutic trial with antithyroid drugs but this procedure is hardly justified today.

e) Treatment of Graves' Disease

Even mild hyperthyroidism due to Graves' disease should always be treated. Three basic forms of treatment are available:

antithyroid drugs,
subtotal thyroidectomy,
radioiodine.

All three effectively reduce the production of thyroid hormones. In recent years it has become possible to enhance the effectiveness and the rapidity of onset of the above treatments by sympatholytic drugs, which block some of the peripheral manifestations of thyrotoxicosis without reducing the secretion of thyroid hormone per se.

Although we shall attempt to give certain guidelines, the choice of treatment cannot be rigidly prescribed, since it is the subject of heated debate even among authorities.

In some centers, particularly in the U.S.A., most patients are treated with radioiodine. In Europe, including the United Kingdom, patients are more evenly distributed among thyroidectomy, antithyroid drug therapy and radioiodine. Several factors, including the system of medical care in the population, are undoubtedly responsible for the differences. Radioiodine treatment does not require admission to hospital and only infrequent follow-up visits are necessary. This may be one reason for its popularity in the U.S.A., where the enormous costs of hospital admission are not borne by a national health service. Another source of bias is the referral practice of the family doctors. Patients referred to a nuclear medicine department or to a surgical service will obviously be much more likely to obtain the specific form of treatment in which the given service specializes.

α) Antithyroid Drugs

Thionamide Drugs are the most frequently used. They are all derivatives of thiourea, which is itself a weak antithyroid drug. Table 6 lists the three preparations currently most prescribed, propyl-(or methyl-)thiouracil, carbimazole and methimazole, together with their mean dosage. Fig. 21 gives their chemical formulas.

Thionamide drugs inhibit the incorporation of iodide into the organic compounds of thyroglobulin, i.e. they interfere with the formation of mono- and diiodotyrosyl residues, the two precursors of the thyroid hormones (Fig. 3, p. 139). As outlined on p. 140, organic iodination is catalyzed by a peroxidase which uses hydrogen peroxide and iodide as substrates. Since the exact molecular mechanism of the iodination reaction has not yet been worked out, the mode of action of thionamide drugs is not known. The drugs themselves are reducing agents, and they may act simply by reducing the oxidized iodine intermediate (I_2 or I^+) as rapidly as it is formed by the peroxidase. Other possible mechanisms have been discussed by ASTWOOD (1955). In an exhaustive review, MALOOF (1963) has suggested that the iodinating intermediate is actually sulfenyl iodide ($-S-I$) attached to a cysteyl residue of a protein. Thionamide drugs may inhibit the formation of sulfenyl iodides by forming mixed disulfides with the SH groups of the protein involved.

There are indications that thionamide drugs also inhibit a further step of hormone synthesis, namely the coupling reaction (p. 141). This effect is more difficult to demonstrate experimentally.

MORREALE DE ESCOBAR (1967) has pointed out that propylthiouracil inhibits the peripheral deiodination of thyroxine by an extrathyroidal effect in rat. Whether this effect also occurs in man is not yet known (BINSWANGER, 1966; FURTH, 1966). Very recently OPPENHEIMER (1972) has amply confirmed these findings and added evidence that propylthiouracil blocks the peripheral conversion of thyroxine to triiodothyronine. Since he considers triiodothyronine the only active form of thyroid hormone, these observations are possibly of great clinical importance. In a recent study, VAN PILSUM (1973) found that methimazole markedly reduced the effectiveness of thyroxine replacement in thyroidectomized rats.

Oral methimazole is rapidly absorbed in man and peak plasma levels are reached 30 min after ingestion; the serum half-life varies from 150–360 min. The drug is accumulated in the thyroid and the adrenal glands (PITTMAN, 1971). Carbimazole is rapidly converted into methimazole and accumulated in this form in the thyroid gland (MARCHANT, 1972b). The sulfur atom of ^{35}S-labeled methimazole is rapidly oxidized to sulfate (MARCHANT, 1972a).

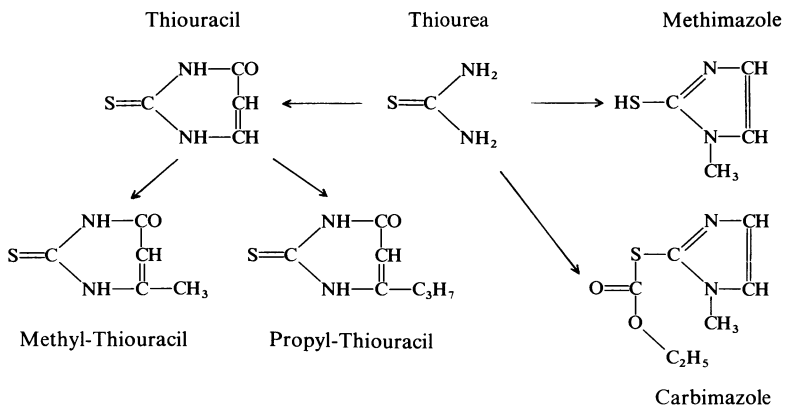


Fig. 21. Chemical formulas of the most common antithyroid drugs

We use the following indications for administration of thionamide drugs:

1. As a preparation for subtotal thyroidectomy, often in conjunction with iodine (p. 193);
2. In Graves' disease in children or adolescents;
3. In Graves' disease in pregnancy, when subtotal thyroidectomy is not possible;
4. Occasionally after radioiodine treatment to hasten the attainment of euthyroidism, if this is necessary.

Other endocrinologists accept much wider indications for antithyroid drug therapy, and use it as the treatment of choice in all cases where it is not contraindicated (VANDERLAAN, 1972). Hypersensitivity to the drug is an absolute contraindication. Relative contraindications are large goiters (long-term results are usually poor and the goiter may grow and cause pressure symptoms during treatment), relapse after a previous full course of antithyroid drugs or after subtotal thyroidectomy, and unreliability of the patient or impossibility of frequent follow-up visits.

There is general agreement that if one decides upon drug therapy of Graves' disease, the treatment should last for at least 12 months. It is not known whether the drugs alter the basic disease process in any way, and actively induce remission. From the few data available on the natural course of the disease we rather suspect that thionamide drugs allow the patient to be maintained in an artificially euthyroid state while waiting for a spontaneous remission (p. 188).

The initial dose should be relatively high and divided into three 8-hourly doses (Table 6). As soon as signs of improvement appear the dose is rapidly tapered and the maintenance dose established by trial and error. In many cases it is possible to maintain treatment by a single daily dose (GREER, 1965). Weekly checkups are necessary at first. Later the patient should be seen every two to four weeks. The clinical state is usually an adequate indication of the effect of treatment, but the total serum thyroxine should be measured at intervals to confirm the clinical findings. The usual radioiodine uptake test cannot be carried out during drug treatment. Leukocyte counts should be obtained at each patient visit, although this is not an infallible method of avoiding agranulocytosis, since this complication may develop very suddenly.

The patient usually notes some improvement after one week of treatment, and euthyroidism is reached after 4–10 weeks (CHEVALLEY, 1954). The onset of action is slow because thionamide drugs block the biosynthesis but not the release of thyroid hormone. Hormone secretion can therefore continue until the relatively large hormone stores are exhausted (p. 141). Thionamide drugs interfere with organic binding of iodide and thus greatly inhibit the thyroidal uptake of ^{131}I when measured at the conventional 2- and 24-hour intervals. However, they have little influence on the 10- or 20-minute uptake, which represents mostly iodide transport across the cell membrane (p. 139). Since this transport is under the control of TSH, it decreases when triiodothyronine is given to a normal person. In Graves' disease triiodothyronine does not have this effect, since the thyroid gland operates independently of TSH control. This property has been used by ALEXANDER (1970) to decide whether a patient under treatment has entered remission, in which case the drug may be stopped with no danger of relapse. Although the test did not allow him to ascertain the outcome in every case, it did statistically separate the group of relapse-prone patients from the group of patients that remained in remission. Of the "suppressed" patients, only 31% had a subsequent relapse, while 76% of the "nonsuppressed" patients relapsed. CASSIDY (1970) and LOWRY (1971) have published similar results. Since the test does not completely separate the relapse-prone patients from those going into remission, and since it is not simple, we do not recommend it for routine use. We think it is as well to stop treatment after 12 months and then wait and see whether the disease relapses. CHOPRA (1970) has studied the correlation between the presence of LATS and the nonsuppressibility of thyroid function. His failure to find any such correlation

Table 6. Dosage of antithyroid agents. Dose in mg/day

	Methyl- or propylthiouracil	Methimazole, carbimazole	Perchlorate[a]
Initial dose during first 10 days (divided into three 8-hourly doses)	300–400	30–60	1000–1500
Maintenance dose (given in one or two daily doses)	25–100	2.5–15	200–600

[a] The routine use of perchlorate is not recommended, except in special circumstances (see text).

is a strong argument against the causative role of LATS in Graves' disease.

Recurrence, unfortunately, is quite frequent. In HERSHMAN'S (1966) series of 176 patients with a follow-up of 6 to 23 years 45% had a recurrence, which appeared within the first year after discontinuation of the drugs in most cases. ALEXANDER (1973) has published essentially similar results. In a study from Japan, SHIZUME (1970) has confirmed the high relapse rate. He found the rate of permanent remission was high when the following features were present: 1. Small goiter; 2. Short duration of symptoms; 3. Reduction in goiter size during drug treatment; 4. Positive suppression test at the conclusion of the drug treatment.

A *relapse* after drug treatment should *not* usually be treated by a second course of drugs, because the chance of a second relapse is more than 75% (MCLARTY, 1969). Patients in relapse should therefore undergo ablative therapy, whether with radioiodine or by surgery. The latter approach led to satisfactory control of hyperthyroidism in most of MCLARTY'S cases, but produced an unusually high incidence of surgical complications (MCLARTY, 1969). A few authors prefer to treat relapses by continuous drug administration, if necessary over 20 years (VAN DER LAAN, 1972).

Side effects of thionamide drugs occasionally necessitate discontinuation of treatment. The drugs are inherently goitrogenic in normal persons and animals, because they interfere with thyroid hormone synthesis. TSH secretion is thus enhanced, and TSH in turn produces growth of the thyroid gland. In the treatment of Graves' disease the goitrogenic effect is rarely a problem, because TSH secretion is suppressed anyway as long as the thyroid hormone level in the blood is elevated. Growth of the gland under drug treatment is usually a sign that the patient has become hypothyroid and the dose should be reduced. Some authors prefer to continue on the high drug doses in such cases and to add thyroxine treatment to suppress TSH. However, we do not think that this procedure is justified, since it is desirable to give the lowest effective dose of thionamides (see below).

Skin rashes, pruritus, urticarial febrile reactions, drug fever and granulocytopenia are occasionally observed. In a series of 184 patients treated with methimazole, CHEVALLEY (1954) observed skin rashes, urticaria or fever in 5 and granulocytopenia in 3. Granulocytopenia was reversible in all 3 cases. The incidence of complications was dose-related and rose steeply at a daily dose of more than 40 mg, an observation confirmed by BARTELS (1952) and recently by WIBERG (1972), who observed toxic reactions in 9 out of 25 patients treated with methimazole in a dose of 120 mg daily. In the studies the drug was given to most patients for a short period of a few weeks to a few months. Reports on the toxicity during a one-year treatment are not available. Agranulocytosis is a much-dreaded complication of treatment, but fortunately it is very rare with the usual dose. Cholestatic jaundice has been reported in a few isolated cases (FISCHER, 1973). Mild toxic reactions sometimes disappear on dose reduction, but it is usually necessary to discontinue treatment. A switch from one thionamide drug to another may be tried (e.g. from methimazole to propylthiouracil), but there is often cross-sensitivity. Since carbimazole is rapidly converted within the body into methimazole (MARCHANT, 1972b), there is little point in changing from one of these drugs to the other. In rat, prolonged treatment with thionamides has produced thyroid adenomas and rarely carcinomas. The histological changes produced in man by antithyroid drugs have been analyzed by WEGELIN (1948).

Perchlorate has been successfully used in the treatment of Graves' disease (GODLEY, 1945; MORGANS, 1954), but due to the high incidence of toxic reactions (KREVANS, 1962; JOHNSON, 1965) it has never become popular. The mechanism of action differs from that of thionamides, since it inhibits iodide transport across the thyroid cell membrane (p. 139). We use perchlorate only for diagnostic purposes in cases of suspected peroxidase deficiency (p. 243) and in the very rare instances of hypersensitivity to all thionamide compounds where surgery and radioiodine treatment are also not feasible.

In recent studies, *lithium carbonate* has shown great promise as an antithyroid agent (TEMPLE, 1972). Its mode of action is complex, but it seems more or less established that it inhibits hormone release, probably by interfering with colloid droplet formation. It therefore has a much more rapid onset of action than the thionamides and it approaches iodide in its efficacy (see below). Due to its side effects, which make continuous monitoring of blood levels mandatory, it cannot yet be recommended for routine use (see also p. 154).

Pharmacological doses of iodide were first noted by PLUMMER (1923) to improve symptoms of thyrotoxicosis and to reduce the risk of subtotal thyroidectomy, at that time the only available treatment for Graves' disease. WOLFF (1969) has made a substantial contribution to the understanding of this paradoxical effect of iodide and has masterfully reviewed the literature. The minimal dose of iodide producing an inhibition of thyroid function in man is 5–10 mg/day, which is 100 to 200 times the minimal daily dietary allowance. The inhibitory

serum level in man is approximately 20 µg/100 ml (REINWEIN, 1960). There are several sites of action: 1. Iodide inhibits the formation of organic iodine from iodide. In this respect its site of action is the same as that of thionamides. 2. Iodide inhibits the release of thyroid hormone from preformed hormone stores of the colloid. This unique property makes iodide the antithyroid drug with the most rapid onset of action equalled perhaps only by lithium (TEMPLE, 1972). Indeed WARTOFSKY (1970) has shown that iodide, unlike thionamide drugs, inhibits thyroid hormone secretion immediately in Graves' disease. The site of this iodide effect is not yet known. It is suspected to be either the step of colloid endocytosis or that of lysosomal thyroglobulin hydrolysis (p. 142), but no convincing proof has yet become available. A consequence of this iodide effect is reaccumulation of colloid in colloid-depleted glands in Graves' disease (RAWSON, 1945). 3. Iodide is said to reduce the vascularity in the hyperfunctioning thyroid gland, thereby facilitating surgery. No objective data are available on this effect.

In normal persons the antithyroid effect of iodide is very shortlived and hypothyroidism does not appear despite prolonged treatment e.g. of asthmatics who take iodide as an expectorant. The reasons for this "escape" have been analyzed by BRAVERMAN (1963) and by WOLFF (1969). Iodine excess causes the thyroid gland to shut off active iodide transport by an autoregulatory mechanism ("iodide pump", cf. p. 139). This lowers the intrathyroidal iodide concentration and relieves the gland from the blocking effect of iodide. In thyrotoxicosis this adaptation of the iodide pump does not readily take place for some unknown reason and there is a prolonged block of thyroid function. In NAGATAKI's (1970) study involving 12 patients treated with iodide alone, hyperthyroidism was well controlled in all patients during the first 6 weeks of treatment. However, half the patients "escaped" between the 6th and 14th weeks of therapy despite continued iodide administration. Occasionally, apparently euthyroid patients become hypothyroid when given pharmacological doses of iodide. This so-called iodide myxedema has been discussed on p. 154.

The minimum effective dose of iodide is 5–10 mg/day, but usually much higher doses of 20–200 mg are given to be on the safe side. The preparations most frequently used are saturated solution of potassium iodide (SSKI), which contains 750 mg of iodide/ml, or Lugol's strong iodine solution, which contains 100 mg potassium iodide plus 50 mg iodine (I_2) per ml. The usual dose is 5 drops of SSKI twice daily or 0.5 ml of Lugol's solution twice daily per os. In most cases it is useless to give larger doses (FRIEND, 1960). If necessary potassium iodide may be given in a slow intravenous infusion over 24 hours. In most cases iodide is given in combination with thionamides, but it should never be given with perchlorate as this abolishes the iodide effect.

β) Subtotal Thyroidectomy

Until the advent of antithyroid drugs, subtotal thyroidectomy was the only available form of treatment, but it was a very dangerous procedure with a high incidence of postoperative thyroid crisis and a high mortality. Many surgeons therefore preferred to remove a small part of the gland in each of several operations, or to limit the operation to ligation of the thyroid arteries. This grim outlook was dramatically changed when PLUMMER (1923) introduced iodine into the preoperative treatment of Graves' disease (p. 192). With proper management the operative mortality is now near zero (MCNEILL, 1968). We use the following indications for the operation: 1. Patients under 25 who are not adequately controlled by antithyroid drugs (the age limit is arbitrary; many centers use higher limits, and a few go as low as 20 years); 2. Patients with large nodular goiters causing pressure symptoms; 3. Patients with fast-growing firm goiters and suspected malignancy. Previous thyroid surgery is a relative contraindication, since the results of a second operation are very poor (MCLARTY, 1969; HEDLEY, 1970a). Many consider severe ophthalmopathy a contraindication, but the connection between thyroidectomy and exacerbation of ocular changes is not universally accepted.

The important point in the preoperative management is that *the patient be handed over to the surgeon in the euthyroid state*. This is usually achieved by giving a thionamide drug (e.g. carbimazole or methimazole in a dose of 45 mg per day) over two to four weeks. When the patient is clinically euthyroid and the serum thyroxine is in the normal range he may be subjected to surgery. Iodine therapy can be given in conjunction with thionamides (p. 192) with two aims in mind: 1. acceleration of the appearance of euthyroidism; 2. Reduction of the vascularity of the gland, which is said to make the operation easier. However this latter iodine effect is not undisputed and we therefore do not give iodine routinely unless the surgeon specifically wishes us to. To be on the safe side the drugs should also be given for 3 or 4 days after surgery, but they may then be rapidly

tapered and discontinued altogether. If exceptional circumstances make it necessary to operate on a patient who is not yet completely euthyroid, anesthesia must be induced and maintained with great care. Hyperthyroid patients are very sensitive to sympathomimetic drugs and to atropine, which should be given in low doses or replaced by scopolamine. The use of ether and nitous oxide in preference to cyclopropane or fluothane is recommended.

The internationally accepted procedure is subtotal intracapsular thyroidectomy. A remnant of 4 to 10 g is left lining the posterior face of the capsule. Since the parathyroid glands are usually located in this part of the thyroid they are left intact by this procedure in most cases. The incidence of complications is low when the operation is performed by an experienced surgeon. Permanent hypoparathyroidism develops in 1–2% of cases in most series (DAVIS, 1961; MCNEIL, 1968). Transient hypoparathyroidism and latent hypoparathyroidism may be more frequent. Transient postoperative hypocalcemia does not necessarily mean hypoparathyroidism, since it is often due to a greatly increased calcium uptake by the osteoporotic bones (MICHIE, 1971).

The obvious explanation for the effectiveness of the operation is that it greatly reduces the number of active thyroid cells. Lately, however, HEDLEY (1971) has made the provocative suggestion that the operation improves the underlying autoimmune disease. This contention is based on the lower incidence of LATS in postoperative patients. His results are subject to the drawbacks of any retrospective set-up and will therefore have to be confirmed by additional studies.

The incidence of postoperative hypothyroidism seems to be closely related to the weight of the thyroid remnant (MICHIE, 1972). It may therefore be as low as 6.5% (MCNEILL) or as high as 49% (MICHIE, 1972), depending on the surgeon's policy. Most patients become hypothyroid within 4 months of the operation and most authors have found no evidence of a cumulative rise comparable to that after radioiodine treatment (MICHIE, 1972; HEDLEY, 1970a). In one study, however, there was a cumulative rise of hypothyroidism of 1.7% per year over 10 years (NOFAL, 1966). There may be a positive correlation between the presence of thyroid antibodies in serum (BUCHANAN, 1962) or the degree of lymphocytic infiltration of the gland (HARGREAVES, 1968) and the occurrence of postoperative hypothyroidism, but this point remains to be confirmed. Diminished thyroid reserve (as defined on p. 161) may be present in many patients who are capable of maintaining a euthyroid state only thanks to elevated TSH levels (HEDLEY, 1970b).

Recurrent thyrotoxicosis is found after surgery in 2–30% of patients (see HEDLEY, 1971, for short review). The treatment of choice for postoperative relapse is radioiodine, since both a second operation and antithyroid drugs are ineffective in controlling the disease in most cases (MCLARTY, 1969; HEDLEY, 1970a).

γ) Radioiodine

Radioiodine may be administered either per os or intravenously. In Graves' disease 30–90% of the radioactivity will rapidly be taken up by the hyperfunctioning thyroid gland, while the remainder will be excreted in the urine. The radioiodine is slowly lost from the gland into the blood as labeled hormone. The most frequently used iodine isotope, ^{131}I, has a physical half-life of 8 days. Its decay produces both β-particles with a mean tissue pathway of about 1 mm and γ-quanta. The β-particles are responsible for 90% of the therapeutic effect; on being absorbed they destroy thyroid tissue without affecting neighboring organs. The γ-ray emission of ^{131}I is used mainly for diagnostic purposes. The chemical amount of iodine introduced into the body by radioiodine treatment is so small that it has no biochemical or pharmacological effect (1 mCi of carrier-free ^{131}I = 0.01 μg iodine).

Indications for radioiodine vary from one center to the other. In some clinics radioiodine is used whenever it is not contraindicated and 80% or more of patients with Graves' disease are treated in this way. As far as risks (except hypothyroidism), costs, and inconvenience to the patient are concerned, radioiodine is indeed unsurpassed by any other treatment for Graves' disease.

There are several contraindications to the use of radioiodine. Since the fetal thyroid accumulates iodine from the third month of gestation onwards, radioiodine should never be given during pregnancy. Radioiodine is excreted into the milk and should therefore not be given to a nursing mother.

The thyroid gland appears to be very sensitive to the carcinogenic effect of radiation in childhood. Radioiodine should therefore not be given to patients under the age of 25 (this is an arbitrary limit). Relative contraindications are large diffuse and large nodular goiters with rapid radioiodine turnover, since unduly high doses of radioactivity have to be applied, producing whole-body irradiation in excess of permissible limits.

Strong *indications* for radioiodine are:

1. Recurrent hyperthyroidism after thyroidectomy. In these cases radioiodine is the treatment of choice (p. 194);
2. Recurrent hyperthyroidism after drug treatment. In these cases the options are radioiodine or surgery;
3. Severe ophthalmopathy. Radioiodine is often preferred in these cases, but reliable evidence that this form of treatment causes less progression of the eye disease is hard to come by.

We consider as *relative indications* all cases of established Graves' disease in patients over age 25, whether with diffuse or with nodular goiter.

There are several potential *risks* in radioiodine therapy:

1. Carcinogenic effect on the thyroid tissue;
2. Production of leukemia due to bone marrow irradiation;
3. Production of recessive mutations due to gonadal irradiation;
4. Hypothyroidism.

All these risks have to be weighed against those of surgery or drug treatment. It is now safe to say that the first three potential risks listed above are negligible, while the fourth, hypothyroidism, remains a real problem. Although isolated cases of thyroid neoplasia after radioiodine treatment have occurred (SHELINE, 1962; BAKER, 1969; LIMA, 1970), it is generally agreed that the incidence is extremely low, and probably no higher than in a control population. MCDOUGALL (1971) has recently reviewed the literature on this subject. Chromosomal changes have been found in the lymphocytes of radioiodine-treated patients (CANTOLINO, 1966), but in two very carefully followed series of 10000 and 20000 patients the incidence of leukemia was no higher than in the general population (POCHIN, 1960; SAENGER, 1968).

The radiation dose received by the gonads in the course of radioiodine treatment (about 20–30 rad) is in the same order of magnitude as the dose received during a gastrointestinal radiologic examination. The genetic risk to the population induced by radioiodine is only a tiny fraction of that produced by radiation from natural sources, and the expected rate of mutations in the descendants of radioiodine-treated parents is only minimally higher than in untreated parents. We agree therefore with HORST (1960) that, with the exception of Graves' disease in childhood, the indication for radioiodine treatment should be made dependent on the calculated whole-body dose rather than on an arbitrary age limit.

The report of DUNN (1964) has drawn attention to the alarmingly high incidence of postradioiodine hypothyroidism, a phenomenon which has been well studied in recent years. In DUNN'S report, 20% of patients were hypothyroid after one year and the incidence of hypothyroidism subsequently rose by about 2.5% per year to reach 45% after 14 years There was no indication that this rise levelled off. In another series 70% were hypothyroid after 10 years (NOFAL, 1966). There is no doubt that the incidence of hypothyroidism is related to the dose of radioiodine (HAGEN, 1967; SMITH, 1967; GOOLDEN, 1969), but GLENNON (1972) has recently examined 55 patients treated before 1951 with very low doses of 3 mCi or less. Although the incidence of hypothyroidism remained quite low for 5 years, it then started to rise at a rate of 3.4% per year, reaching a cumulative incidence of 48% after 17 years. Glennon suggested that the dose influenced the incidence of "early" hypothyroidism during the first years, but that the subsequent cumulative rise was independent of the amount of ^{131}I. In Zurich, with the dosage calculated as outlined below, the rate of increase of hypothyroidism was 1.6% per year (ENGELBRECHT, 1970). According to more recent results from the same group (KAESTNER, 1973) this low incidence may be due to the fact that their sample included an appreciable number of patients suffering from Graves' disease with nodular goiters. Indeed, patients with nodular goiters appeared to be much more resistant to the development of hypothyroidism than those with diffuse goiters (1.6% as against 14.7% at 5 years). A similar difference had already been found by VIHERKOSKI (1970), but toxic adenomas were not rigorously excluded in his series.

The exact mode of action of ^{131}I has not yet been established, but it is generally assumed that it damages the thyroid cell nucleus and thereby interferes with cell division (GREIG, 1965). Since there is probably a slow turnover of cells, inhibition of cell replication will progessively diminish the number of active cells. In order to avoid nuclear irradiation, another iodine isotope, ^{125}I, has recently been used in experimental studies. This isotope has a half-life of 60 days. It has a complex decay scheme with the energy dissipated by γ-emission (7%) or by internal conversion (93%). The latter produces soft X-rays or low-energy β-particles, both with a very short tissue pathway of about 0.5 μm. Since most of the radioactive iodine in the thyroid gland is within the colloid, the apical cell pole receives most of the radiation, due to the short pathway, while the basal cell pole, which contains the nucleus, is left intact.

Several groups of investigators have therefore used ^{125}I for the treatment of Graves' disease, and th theoretic and practical aspects have been c ncisely reviewed by McDougall (1971) and also in an Editorial in the Lancet (1972). The studies so far have shown that ^{125}I effectively controls hyperthyroidism, but with respect to hypothyroidism the results have been rather disappointing, since the percentage appears to be as high as or even higher than after ^{131}I (McDougall, 1971; Werner, 1970). At present, therefore, ^{125}I remains an experimental form of treatment.

The high risk of hypothyroidism after radioiodine places a great responsibility upon the doctor. First of all he has to inform the patient of this risk and to tell him about the most common symptoms of hypothyroidism. Second, the patient *must be followed up for the rest of his life* and examined by the doctor at least once each year. Failure of the patient to keep his appointment should make the doctor suspect that he has become hypothyroid, and every effort should be made to contact him and to bring him under medical supervision. It is the fact that hypothyroidism is latent for some years, develops insidiously, and may easily be mistaken for cerebral arteriosclerosis in older persons that makes this lifelong follow-up mandatory. Regional follow-up schemes have been devised in Scotland (Philp, 1968). Once it is diagnosed, hypothyroidism is so easy and cheap to treat that in our view its occurrence is no reason not to use ^{131}I.

Numerous methods are in use for the calculation of the *dose of* ^{131}I. All the methods use at least one variable, the estimated thyroid weight, in the computation. Thus, in most centers in the United States the gland weight is estimated by several observers by palpation and 80–160 μCi of ^{131}I per g weight are given. The calculations used in Zurich include several other variables, in an attempt at more exact control of the radiation energy delivered to the tissue. The following unit is in use as a measure of the radiation energy:

rad (radiation absorbed dose): 1 rad corresponds to 100 erg of energy delivered to 1 g of tissue.*

The following 5 factors or variables are considered in calculations of dosage for individual cases:

1. Physical properties of ^{131}I: The β-emission energy, which is the one relevant to its therapeutic effect, is 0.205 MeV, including conversion electrons (Marinelli, 1948). With additional components of the isotopic decay, in particular γ-emission, the total energy is 0.248 MeV (Perry, 1950). One mCi of ^{131}I delivers 0.56 rad/hour in 1 g of tissue, provided the isotope distribution is homogeneous.

2. Volume or weight of the thyroid gland, which may be estimated by palpation or by planimetry of thyroid scintigrams. We use the following formula: Weight = 0.323 × average height of lobes × surface of gland in a dorsoventral scintiscan (Allen, 1952; Doering, 1958). The mean deviation of this estimation from the weight found at autopsy was 10% (Gauwerky, 1959).

3. Percentage or fraction of the ^{131}I dose taken up by the thyroid. Several measurements must be taken after a diagnostic dose to obtain the peak uptake.

4. Distribution of ^{131}I *within the thyroid tissue.* Since radioiodine is unevenly distributed in thyroid tissue (Levene, 1955) the resultant intrathyroidal dose lattice is nonuniform. Dose maxima at certain sites can be 3.4 to 25 times greater (mean 10 times greater) than the calculated average dose (Sinclair, 1956). The dose minima may be quite important, since they may allow the survival of normal thyroid tissue which will maintain euthyroidism.

Gross inhomogeneity of radioiodine distribution such as occurs in nodular goiters usually calls for an increase in the dose of ^{131}I.

5. Rate of release of radioiodine from the thyroid gland. This is included in the final formula as effective half-life (T_{eff}), which is obtained from the equation:

$$T_{eff} = \frac{T_{biol} \times T_{phys}}{T_{biol} + T_{phys}}$$

where T_{biol} is the biologic and T_{phys} the physical half-life. The biologic half-life can be determined directly from serial epithyroidal measurements of radioactivity or estimated from the PB ^{131}I, which correlates closely with T_{biol}.

The final formula used in Zurich for the dose in μCi ^{131}I (D) is:

$$D = \frac{W \times \text{rad}}{19.4 \times T_{eff} \times U}$$ (modified from Oeser, 1961)

where W = thyroid weight (grams), T_{eff} = effective half-life (days), U = thyroid uptake of ^{131}I (fraction of dose), rad = the desired radiation dose, as outlined in Table 7. The factor 19.4 contains several simple transformations (hours to days, rep to rad, half-life into mean life).

We vary the radiation dose according to Table 8, e.g. grade I in patients with severe exophthalmos in whom high doses might ag-

* 1 rad ≙ 1.07 rep (roentgen equivalent, physical).

Table 7. Guidelines for radioiodine dosage in Graves' disease (SCHNEIDER, 1965). (See text for explanation)

	I	II	III	
Very small goiter	3000	5000	7000	rep
Goiter < 120 g	4000	7000	10000	rep
Goiter > 120 g	5000	9000	12000	rep

Table 8. Indications and contraindications for various forms of treatment of thyrotoxicosis

1. Radioiodine
   a) Never during pregnancy;
   b) Never under age 25, except in a few special cases;
   c) Treatment of choice for relapse after operation.
2. Subtotal thyroidectomy
   a) Always in rapidly growing goiters with suspected malignancy;
   b) Often in large nodular goiters with pressure symptoms (radioiodine may be an alternative).
3. Thionamide drugs
   a) Always preoperatively before thyroidectomy, often in conjunction with iodine;
   b) In all cases of Graves' disease during childhood;
   c) Always in thyrotoxic crisis, in combination with iodine;
   d) In most cases during pregnancy (thyroidectomy is an alternative). Never give iodide during pregnancy;
   e) Sometimes as an adjunct after radioiodine to hasten appearance of euthyroidism;
   f) In selected adult patients as a 1-year course;
   g) Not recommended in cases of relapse after a first course of drugs or after surgery.

gravate the eye problem and grade III in elderly patients with heart failure, in whom rapid control of thyrotoxicosis is desirable.

The results of radioiodine treatment are usually quite satisfactory if the high risk of hypothyroidism is disregarded. Thyrotoxicosis is controlled within 3 to 6 months in most cases. After this time a second dose can be given if needed. The slow onset of action is a disadvantage, but control is obtained more quickly by beginning administration of a thionamide drug or iodine (HAGEN, 1967) 10–14 days after the ^{131}I dose.

Quite a number of patients are pretreated with thionamide drugs before the decision to use ^{131}I is made; this is then usually given two to three weeks after the drug has been stopped. GOOLDEN (1969) found that in such cases pretreatment with methimazole or propylthiouracil lowered the cure rate of ^{131}I, while carbimazole had no such effect.

Some patients show a transitory exacerbation of thyrotoxic symptoms, and LAMBERG (1959) and VIHERKOSKI (1970) have stressed the danger of thyrotoxic crisis after ^{131}I. In our experience, however, this is a very rare complication. A few patients complain of pain in the neck region between the second and tenth day after ^{131}I.

δ) Ancillary Treatment: Antiadrenergic Agents, Sedatives

The primary aim in the treatment of thyrotoxicosis is the reduction of thyroid hormone secretion. However, in recent years drugs have become available which decrease the peripheral effects of thyroid hormone excess and they have been successfully used in cases where rapid control of disturbing or dangerous signs or symptoms is desirable. We would like to emphasize at the onset that although the patient may be greatly improved by such treatment, this does *not* render treatment directed at the thyroid gland itself unnecessary.

The drugs most frequently used are the sympatholytic agent guanethidine, the β-blocking agent propranolol, and the α-blocking agent phentolamine, or a combination of these (STOUT, 1969; BECKER, 1972). In double blind trials β-blocking agents successfully improved several signs and symptoms of hyperthyroidism, such as palpitations, nervousness, tachycardia, tremor, perspiration, and shortening of the ankle jerk (SHANKS, 1969; GROSSMAN, 1971). The usual oral dose of propranolol is 40–160 mg per day. Propranolol does not interfere with any of the thyroid function tests except the ankle jerk; in particilar it does not affect radioiodine uptake (HADDEN, 1969), so that it can be given in conjunction with radioiodine. Although propranolol affords good relief of many thyrotoxic symptoms, it should not be given alone, since only few patients are satisfactorily controlled (MCLARTY, 1973).

An important question is whether β-blocking agents may be given to thyrotoxic patients with heart failure. This will be discussed in the section on thyroid crisis (p. 198), a situation in which these agents are now of great importance.

In agitated patients phenobarbital or diazepam may be prescribed. Diazepam has been specifically examined and found *not* to interfere with thyroid function tests (CLARK, 1971).

ε) Choice of Treatment

Our guidelines for the indications for the various forms of treatment are summarized in Table 8, and the advantages or disadvantages of each procedure are compared in Table 9.

f) Special Forms and Complications of Graves' Disease

α) Thyrotoxic Crisis (Thyroid Storm)

This is a much-dreaded complication of Graves' disease. MCARTHUR (1947) has defined it as

Table 9. Advantages and disadvantages of the various forms of treatment

	Antithyroid drugs	Radioiodine	Subtotal thyroidectomy
Reliability of antithyroid effect	Very good	Good	Good
Time required to reach euthyroidism	4–8 weeks	3 months	1 week
Cost	Moderate	Low	High
Hospital stay	No	No	Yes
Follow-up visits	Every month	Every 6 months	Every year
Risk of permanent hypothyroidism	0	25–70%	6–50%
Risk of relapse	50%	10%	6–25%
Mortality	<1%	0	<1%

a *potentially fatal exacerbation of all thyrotoxic symptoms.* An elevation of rectal body temperature to at least 38.8 °C is considered an obligatory sign. The exact cause of thyrotoxic crisis is unknown, but a sudden release of massive amounts of thyroid hormones is probably implicated. We recently observed one case with a serum thyroxine concentration of 50 µg/100 ml. Some authors contend that thyroid crisis often occurs in patients whose serum hormone level is no more elevated than in ordinary thyrotoxicosis. They therefore think that massive thyroid hormone excess is not the decisive factor in the pathogenesis (DILLON, 1970), but we suspect their opinion may have to be reevaluated when triiodothyronine concentrations during thyroid crisis become known.

Among 2033 thyrotoxic inpatients admitted to the Massachussetts General Hospital between 1921 and 1946, MCARTHUR (1947) found 36 cases of thyrotoxic crisis. In 25 patients the crisis was "surgical", i.e. it occurred after subtotal thyroidectomy. In the remaining 11 patients the crisis was classified as "medical" i.e. occurring after infection, trauma or anesthesia. As Graves' disease is now more often correctly diagnosed and treated thyroid crisis has become quite rare.

LAMBERG (1959) has carefully analyzed the symptomatology. Most symptoms involve the cardiovascular or the central nervous system, or both. In the so-called *cerebrobulbar* form the patient becomes agitated, confused, or sometimes comatose (ROIZEN, 1971). Bulbar palsy may become a prominent symptom. In the *cardiac* form tachycardia, auricular fibrillation, heart failure, pulmonary edema and shock predominate. *Gastrointestinal* signs with diarrhea are often present, sometimes as an isolated feature. The skin of the patient is red and hot. There is massive perspiration and the body temperature may rapidly rise to 40 °C or more. Hypokalemia with peripheral palsy has been described (LOGOTHETIS, 1962).

Without proper treatment, the crisis is lethal in 100% of cases (LAHEY, 1928). In recent series the mortality has been lowered to 60% (LAMBERG, 1959) and even to 28% (WALDSTEIN, 1960). This is probably due to two factors: 1. Earlier recognition of the crisis, and 2. Availability of personnel trained in intensive care and availability of potent antithyroid drugs.

Treatment of thyrotoxic crisis must begin without delay (Table 10). Blood should be taken for electrolyte determinations and for thyroid hormone measurements, and treatment should be started without waiting for the results of the latter tests. Whenever possible patients should be placed in an intensive care unit

Table 10. Synopsis of clinical features and treatment of thyrotoxic crisis.

Clinical findings

Increase in signs and symptoms of thyrotoxicosis
Fever with temperature over 38° C up to 41° C or more
Perspiration, later dehydration
Agitation, confusion, coma
Bulbar palsy (particularly IXth nerve)
Tachycardia, heart failure

Treatment

Blood should be taken for thyroid function tests, but treatment must *not* be delayed until the results are available.

1. Antithyroid agents (always give a *and* b)
   a) Methimazole or carbimazole 30 mg every 6 hours p.o. or by gastric tube. If available, methimazole (Favistan®) can be given intravenously.
   b) Iodine: Saturated solution of potassium iodide 3 drops every 6 hours or Lugol's strong iodine solution 1 ml every 6 hours. (If necessary: Potassium iodide 0.5 g in 5% glucose or 0.9% NaCl by slow i.v. infusion over 24 hours).
2. Fluid and electrolyte replacement, based on monitoring of central venous pressure and 12-hourly serum electrolyte measurements.
3. In cases of severe fever cooling blankets or "surgical" hypothermia.
4. Sedation: Phenobarbital, diazepam, chlorpromazine, reserpine, alone or in combination.
5. Oxygen 2 liters per minute by nasal catheter.
6. Propanolol 40 to 80 mg p.o. every 6 hours or 1/8 this dose i.v. In older patients and cases of severe heart failure lower doses should be tried first.
7. Digitalis glycosides in relatively high doses (exact dosage guidelines not available).
8. Cortisone acetate p.o. or Cortisone hemisuccinate i.v. (by slow infusion) 200 mg per 24 hours.
9. Antibiotics if infection suspected.

until the crisis is well under control. Antithyroid drugs must be given in high doses, e.g. methimazole or carbimazole, 30 mg every 6 hours. Due to its rapid effect iodine is still the basis of treatment and should always be added to the above drugs (INGBAR, 1966) in a dose of 500 mg of iodine, either as SSKI or as Lugol's strong iodine solution (see p. 193), given in 4 divided doses. If necessary potassium iodide can be given in an intravenous drip over 24 hours, and if available methimazole can be given by i.v. injection.

Dehydration and electrolyte disturbances are treated by adequate fluid therapy. Since perspiration is greatly increased, large amounts of fluid and NaCl must be replaced. Since heart failure is often present, central venous pressure must be monitored during fluid replacement. In rare cases plasma or albumin solutions are necessary to avoid circulatory collapse.

In cases of heart failure or auricular fibrillation with tachycardia, digitalis glycosides must be given by the intravenous route. The patients usually tolerate large doses, but unfortunately no exact dosage guidelines are available. Oxygen (1 to 2 liters/min) should be given through a nasal catheter.

If hyperthermia threatens to be fatal, cooling blankets (or if not available moist blankets and a fan) should be applied. Sometimes proper surgical hypothermia supervised by an anesthetist must be instituted.

Since relative adrenocortical insufficiency may be involved, we always give large doses of cortisone, either per os or as cortisone hemisuccinate in a slow i.v. infusion over 24 hours (200 to 300 mg per day).

β-Receptor blocking agents are probably a major advance in the treatment of thyrotoxic crisis, but their impact on the outcome has not yet been statistically evaluated. BUCKLE (1968) used β-receptor blockade in two patients who had deteriorated despite all other forms of treatment and noted dramatic improvement. Propranolol is the drug most frequently used. The recommended dosage is 40–80 mg every 6 hours per os or about one eighth of this dose by the i.v. route. Obviously there is some reluctance to use β-adrenergic blockade in older patients where heart failure may coexist. In our limited clinical experience, however, β-adrenergic blockade has improved rather than worsened heart failure, probably by slowing the heart rate and allowing better ventricular filling. Two experimental studies support this view. WIENER (1969) found that propranolol improved the left ventricular efficiency in thyrotoxic subjects, and PIETRAS (1972) reported that the same drug did not increase left ventricular end diastolic pressure in thyrotoxic patients even in cases where it was clearly elevated before propranolol. Before the availability of β-receptor blocking agents guanethidine was widely used.

Infection should be looked for as the possible triggering factor of thyroid storm and in case of suspicion an antibiotic must be given after sampling of blood, sputum and urine for bacteriologic examination. Subtotal thyroidectomy is sometimes advocated but is contraindicated in our opinion.

Most patients in thyrotoxic crisis need large doses of sedatives. Phenobarbital, chlorpromazine, diazepam, and reserpine have all been used either alone or in combinations. DILLON (1971) has successfully used reserpine alone without an antithyroid drug, but we would not recommend this as a routine treatment. Peritoneal dialysis (HERRMANN, 1969) or plasmapheresis (ASHKAR, 1970) effectively removes thyroid hormone from the body, but we still consider them as experimental forms of treatment. A review on the treatment of thyroid storm is given in Table 10.

β) Apathetic Thyrotoxicosis

A case has been made for the classification of patients who instead of being nervous or agitated become quiet, depressed and apathetic into a separate subgroup. The literature on this form of Graves' disease has been reviewed by THOMAS (1970) and in an editorial in the LANCET (1970). Most of these patients are in older age groups and many have cardiac symptoms. In some cases the clinical picture was so misleading that the diagnosis of myxedema was considered (RONNOV, 1973). Thyrotoxicosis is often severe in such cases and may lead to death in heart failure if not treated. Apathetic thyrotoxicosis may even occur in the setting of thyroid storm.

γ) Graves' Disease in Pregnancy

In most cases Graves' disease takes a mild course during pregnancy. There is no evidence that pregnancy aggravates thyrotoxicosis. On the contrary, in many cases pregnancy probably has a beneficial effect on Graves' disease. This may be due to the increased levels of estrogen, which have been shown to ameliorate symptoms of thyrotoxicosis (ZANINOVICH, 1972).

The diagnosis of hyperthyroidism in pregnancy should be made largely on clinical grounds. Radioiodine uptake tests should not be performed in pregnancy. The basal metabolic rate is usually elevated to +20 or +30% at the end of a normal pregnancy and is therefore difficult to interpret.

Serum total thyroxine measurements give elevated values in the second and third trimesters of pregnancy, because estrogens cause an increase of TBG (p. 143). The resin T_3 uptake test in contrast falls into the hypothyroid range.

Iodine is actively accumulated by the fetal thyroid from about the 80th day of gestation onward (GREENBERG, 1970). Radioiodine treatment is therefore strictly contraindicated.

In mild cases of thyrotoxicosis sedatives provide adequate control of the symptoms during pregnancy. Subtotal thyroidectomy after a short course of antithyroid drugs (*without* iodide) is also feasible from the 2nd up to the 6th month.

Thionamide drugs may be given, but they have the disadvantage that they cross the placenta, while the maternal thyroid hormone does not pass to the fetus or only to a limited extent. Thus the drugs may produce fetal hypothyroidism and goiter while the mother is still hyperthyroid. There seems to be no ideal solution to this dilemma in theory, but in practice good results are obtained with the lowest possible dose, e.g. 5–10 mg of methimazole or 50 to 100 mg of propylthiouracil per day. The patient should be left slightly hyperthyroid and the total serum thyroxine should be kept at about 15 µg/100 ml. Some authors recommend the administration of triiodothyronine together with the thionamide drugs in the hope that it may cross the placenta. We can see no theoretic or practical advantage in this procedure.

Pharmacological doses of iodide should never be given, since this procedure involves the risk of fetal and neonatal hypothyroidism, a condition with a grave prognosis (p. 167).

BURROW (1972) has written a short monograph on the interrelation between thyroid gland and pregnancy.

δ) Graves' Disease in Neonates and Children

Hyperthyroidism in the newborn is extremely rare. In most reported cases the mother has also suffered from Graves' disease and had very high LATS titers (MCKENZIE, 1964; SUNSHINE, 1965; GAUTHIER, 1968). As an IgG immunoglobulin, LATS crosses the placenta into the fetal circulation. LATS slowly disappears from the blood of the baby and symptoms of hyperthyroidism abate in parallel without treatment. In a few exceptional cases the disease persists for years (HAYLES, 1972).

In children thyrotoxicosis is quite rare when compared with the incidence in adults. The question as to how it is best treated remains open. Radioiodine is considered contraindicated due to the potential risk of carcinogenesis (p. 195), but its use has nontheless been discussed by SAXENA (1965). In one series of 25 children treated with ^{131}I a papillary carcinoma developed in one.

Subtotal thyroidectomy after drug pretreatment offers the advantage of less frequent relapses, but postoperative myxedema was found in 25% of cases (HAYLES, 1967 and 1972).

After thionamide medication relapses are less frequent in childhood than in adult Graves' disease, and we therefore consider it the treatment of choice in children. The dose is the same as in adults, adjusted according to body surface area. The various aspects of childhood thyrotoxicosis have been reviewed in a short editorial in the British Medical Journal (1965).

ε) Thyrotoxicosis with Excessive T_3 Secretion (T_3-Toxicosis)

Triiodothyronine was discovered in 1953, about 40 years later than thyroxine, and remained neglected for a long time because its concentration in serum was too low to be measured by conventional techniques. With the publication of a method for its measurement STERLING (1969) opened a new era in clinical thyroid research.

Numerous publications have since appeared on this hormone, which LARSEN (1972) has recently reviewed. Due to its higher activity (per mg weight), triiodothyronine plays an important physiologic role and it has even been suggested that thyroxine per se is inactive, unless it is peripherally deiodinated to triiodothyronine (p. 145).

Cases of typical clinical thyrotoxicosis with a normal level of protein-bound iodine in serum (which represents mainly thyroxine) were first recorded a long time ago. By measuring the serum triiodothyronine STERLING (1970) established that these patients suffered from isolated oversecretion of triiodothyronine. In his series, most patients had toxic nodular goiters, but subsequent investigators observed the condition in many cases of Graves' disease (IVY, 1971; HOLLANDER, 1972). There are no clinical features which distinguish thyrotoxicosis due to excess T_3 from the conventional form, and the diagnosis must therefore be made on the basis of triiodothyronine measurements. Sometimes the condition progresses to conventional hyperthyroidism with elevated thyroxine levels (HOLLANDER, 1971). It has also been observed in children (MITSUMA, 1972) and in thyroid carcinoma (SUNG, 1973).

g) Endocrine Ophthalmopathy

α) Definition and Classification

Thyroid hormone excess of any etiology (Graves' disease, toxic nodular goiter, thyrotoxicosis factitia) produces certain nonspecific eye signs, notably lid retraction. This so-called *noninfiltrative* ophthalmopathy is attributed to hyperactivity of the sympathetic nervous system, which is thought to produce contraction of Müller's superior palpebral muscle, but this explanation is not universally accepted.

In contrast, *infiltrative* ophthalmopathy occurs only in patients with Graves' disease, and the severity bears little relation to the degree of thyroid hormone excess. Humoral factors other than thyroid hormone are probably important in its pathogenesis. The American Thyroid Association has recently adopted a classification of the eye changes in Graves' disease, which is presented in its abridged form in Table 11 (WERNER, 1969).

Table 11. Abridged classification of ocular changes of Graves' disease (WERNER, 1969). Each class usually includes the involvements of the preceding class. Each class may be graded mild (a), moderate (b), marked (c) or absent (0). Note that proptosis occurs twice in this classification, first in the mild, benign form of class 1, where it is not considered a bad prognostic sign. When there are additional signs of soft tissue involvement, proptosis becomes part of severe (class 3 to 6) ophthalmopathy

Class	Definition
0	No signs or symptoms
1	Only signs, no symptoms (signs limited to upper lid lag and proptosis)
2	Soft-tissue involvement (symptoms and signs)
3	Proptosis
4	Extra-ocular muscle involvement
5	Corneal involvement
6	Sight loss (optic nerve involvement)

β) Pathogenesis

As stated above, the infiltrative ophthalmopathy bears little relation to thyroid hormone excess and it cannot be produced by the exogenous administration of thyroid hormone. Involvement of both eyes and sometimes the coexistence of related changes in the pretibial skin early suggested that humoral factors played a pathogenic role. When the serum of patients with exophthalmos was injected into some fish species (fundulus, goldfish, carp) or guinea pigs it did in fact produce proptosis and in some assays also an increased sulfur-^{35}S uptake by the so-called Harderian gland. This gland only exists in rodents and is *not* an analog of a lacrimal gland. Rather it corresponds to a part of retro-orbital tissue. The serum factor causing proptosis in the test animal has been termed exophthalmos-producing substance (EPS). EPS may be present in both the serum and the pituitary gland of patients with exophthalmos. In serum of patients with severe ophthalmopathy both very high and low titers have been found by the fish bioassay.

BRUNISH (1962) reported a molecular weight of 40000 for EPS as determined by ultracentrifugation. Whether the EPS of serum is identical with the EPS of the pituitary is open to question (MCKENZIE, 1968). EPS and LATS (p. 179) are certainly not identical. They can be separated in serum by polyvinyl block electrophoresis or by gel filtration. Antibodies to EPS do not inactivate LATS. The physiological significance of EPS has been seriously questioned in recent years. Two important criticisms have been that EPS was tested in a species far removed from man, and that all EPS preparations extracted from the pituitary always contained considerable TSH activity. Recently, however, KOHN (1971) has prepared EPS practically devoid of TSH activity by treating pure bovine TSH with pepsin. He speculated that EPS in thyrotoxicosis could be produced by limited proteolysis of TSH within the pituitary gland. This proteolytic derivative of TSH binds to isolated plasma membranes of retro-orbital tissue. The binding is enhanced by a second humoral factor, presumably a gamma globulin, present in the serum of patients with exophthalmos (WINAND, 1972).

In initial reports LATS was found more frequently and also in higher titers in patients with ophthalmopathy (ADAMS, 1957; WERNER, 1961; KRISS, 1967), and it is probably still correct to say that the incidence of LATS is very high in sera of patients with all three cardinal (thyroidal, ocular, cutaneous) manifestations of Graves' disease (LIPMAN, 1967). However, in a later study, MCKENZIE (1968) found no good correlation between eye changes and the incidence or titer of LATS, an observation which was confirmed by HALL (1970) in euthyroid patients with so-called "ophthalmic Graves' disease" (see below).

By the leukocyte migration test, MAHIEU (1972) found evidence of delayed hypersensitivity to retrobulbar tissue protein in 9 out of 10 patients with progressive exophthalmos, but in none of the controls.

A completely new view of the problem may have been opened up by SEGAL (1973). He observed that an appreciable proportion of manic depressive patients treated with lithium developed a condition undistinguishable from endocrine ophthalmopathy.

In summary, there is some evidence for an autoimmune basis and for a pituitary origin of endocrine ophthalmopathy, but more research is necessary to clarify the etiology (see SMELSER, 1962; WERNER, 1972, for reviews).

γ) Incidence, Pathologic and Clinical Features

Mild eye changes (class I, Fig. 16) are found in about 50% of patients with Graves' disease. Severe involvement (classes 2 to 6, Fig. 17 to 19) is much rarer and has an incidence of about 5% or less. Most reported series of severe ophthalmopathy have a male: female ratio of 1 : 1 or 1 : 2. Since the sex ratio for Graves' disease as a whole is 1 : 5, this points to the fact that male thyrotoxic patients are more prone to the development of serious eye trouble. The involvement is often more severe on one side, although strictly unilateral disease is rare.

In most patients the eye trouble starts at the same time as thyroid hyperfunction, but cases where the ophthalmopathy precedes or follows the onset of hyperthyroidism are by no means rare. In the cases where ophthalmopathy develops in euthyroid patients some discrete changes of thyroid function are often dectected by radioiodine studies. The radioiodine uptake, though within normal limits, often remains nonsuppressible by triiodothyronine administration (IVY, 1972). Also, the $PB^{131}I$ is often elevated, indicating an increased radioiodine turnover. The incidence of thyroglobulin antibodies is quite high in such euthyroid patients. They often have some associated clinical features of Graves' disease, such as goiter, vitiligo or a family history of hyperthyroidism (HALL, 1970).

On histologic examination the mucopolysaccharide and water content of all retrobulbar tissues appears increased. There is more connective fibrous tissue, usually at the expense of adipose tissue. Perivascular infiltrates of mast cells are often seen. All retrobulbar tissues (fat, connective tissue, muscles, lacrimal glands) contain infiltrates of lymphocytes and plasma cells. There is interstitial edema in the extraocular muscles which may progress to degeneration of the muscle fibres with loss of cross striation (see reviews by WEGELIUS, 1957; SMELSER, 1962; RILEY, 1972, see p. 283).

Upper lid retraction (Dalrymple's sign) is the most obvious change in *class I* ophthalmopathy. It is attributed to the increased sympathetic tone causing contraction of Müller's muscle (HAMBURGER, 1972), but since the retraction often persists in patients rendered euthyroid this explanation is not entirely satisfactory. Upper lid retraction may sometimes only become manifest when the patient follows the examiner's finger moving downward in front of him (Graefe's sign). This sign is not very specific. Slight swelling of the lids is often present in class I disease (Fig. 16), but gross swelling (Fig. 18) almost always signifies severe ophthalmopathy (grades 2 to 6).

Protrusion of the eye bulbs (exophthalmos, proptosis) is often present in mild (class I) cases. In severe cases (classes 3 to 6) it can be very mild or sometimes grotesque. It can be measured objectively by the Hertel ophthalmometer, which is applied to both lateral rims of the orbita. The values obtained give the distance from the orbital rim to the apex of the cornea. Values higher than 20 mm represent probable, values above 23 mm proven, exophthalmus. The width of the bony base on which the ophthalmometer reposes should always be recorded to assure reproducible measurements over some time in a given patient. Retraction of the upper lid may simulate the presence of proptosis, but when the lower lid is retracted below the limbus of the cornea and the lower part of the sclera therefore becomes visible, true proptosis is almost always present (Figs. 16 and 17). The cause of the proptosis is an increase in retrobulbar tissue, which sometimes shows myxedematous swelling as well as lymphocytic infiltration. The differential diagnosis of proptosis includes retrobulbar tumors, infiltration of the retrobulbar tissue by lipids in histiocytosis (Hand-Schüller-Christian disease), vascular aneurysms, venous thrombosis, hemangiomas. Some people can push their eyeballs forward voluntarily by contraction of the superior oblique muscles (BERMAN, 1966).

In endocrine exophthalmos the bulbi can be pushed some way back into the orbita by gentle pressure, while such resiliency is absent in cases of retrobulbar tumors. In case of doubt angiography of the carotid artery or the vena angularis is very useful in establishing the presence of a tumor (YASARGIL, 1957; KRAYENBÜHL, 1962). In recent years, ultrasound scanning has proved valuable in the differentiation of endocrine ophthalmopathy from retrobulbar tumors (OSSOINIG, 1969; COLEMAN, 1972). Mild exophthalmos is occasionally present in Cushing's disease (MORGAN, 1958), in liver cirrhosis (SUMMERSKILL, 1968), and in acromegaly. Progressive myopia may occasionally simulate exophthalmos.

Discrete swelling of the eyelids is a frequent finding in patients with Graves' disease. Sometimes the swelling is grotesque and disfiguring (Fig. 18). In addition there may be chemosis, increased lacrimation and a "gritty" feeling.

Hematomas, allergic dermatitis, and leukemic infiltrates all have to be considered in the differential diagnosis of lid swellings.

Moderate and severe forms of ophthalmopathy often involve the extra-ocular muscles. Sometimes the function of only one muscle is impaired (Fig. 19 c), but in severe cases there is total ophthalmoplegia. The disturbance in ocular motility is always myogenic and never due to disease of the central nervous system (ESSLEN, 1961). Paresis of extra-ocular muscles due to other factors may be differentiated by electromyography (HUBER, 1967). On pathological examination the muscles are swollen and densly infiltrated by lymphocytes. The muscle fibers show gross degenerative signs with loss of cross-striation. Occasionally the mucles are transformed into rigid fibrous bands.

In its severest form ophthalmopathy presents with lid swelling, chemosis, protrusion of retrobulbar tissue laterally in the lower lid, quasi total immobility of the bulb, keratitis due to incomplete lid closure (lagophthalmos), and finally progressive deterioration of vision. The latter is usually due to pressure on the optic nerve.

δ) Course and Treatment of Ophthalmopathy

The course of ophthalmopathy is unpredictable and bears little relation to thyroid function. As stated above, the ocular involvement remains mild (class 1) in most patients with Graves' disease. With either drug or radioiodine treatment the proptosis increases slightly over the course of more than three months (KOUTRAS, 1965) and may then stabilize or regress. In a few patients the ophthalmopathy suddenly worsens without apparent cause. This may happen when the patient is hyperthyroid, euthyroid, or more often when he is hypothyroid (HAMILTON, 1967). Abrupt treatment of thyrotoxicosis is thought to worsen the ophthalmopathy (ARANOW, 1965), but HAMILTON (1967) was not able to confirm the widely held belief that subtotal thyroidectomy is a worse offender in this respect than radioiodine. Since it appears essential to avoid iatrogenic hypothyroidism, KOUTRAS (1965) recommended routine prescription of 200 μg of L-thyroxine daily during antithyroid drug or radioiodine treatment of patients with eye changes, but in a subsequent study he found little evidence of any beneficial effect (KOUTRAS, 1970). The erratic spontaneous course and the relatively rare occurrence of severe infiltrative ophthalmopathy render the evaluation of any form of treatment extremely difficult.

Mild forms (class 1) of ophthalmopathy cause no discomfort to the patient and need no specific treatment. Cosmetic problems may arise, especially in women, but they should not be an indication for active treatment, since results are unsatisfactory. The patient should be reassured about the generally benign nature of the eye changes by his doctor.

In severe forms of ophthalmopathy the doctor should carefully talk over the problem with the patient and inform him about the unpredictable course and the available therapy.

Local treatment, although of limited value in changing the underlying disease process, is of prime importance since it may save the patient's eyesight by preventing keratitis. Whichever of the subsequent forms of general treatment is chosen, local treatment should always be given. The patient should wear protective glasses during the day. Eyedrops containing 0.25% methylcellulose should be applied several times daily and also during the night to keep the bulbs moist. When there is incomplete spontaneous lid closure the patient may benefit from adhesive tapes over the lids during the night. Lateral surgical closure of the lids (tarsorrhaphy) may improve lid closure in selected patients. Elevation of the head in bed diminishes accumulation of edema fluid. Diuretics are often prescribed, but their value has never been objectively assessed. CROMBIE (1967) has recommended the use of eyedrops containing 10% guanathidine, and KOUTRAS (1970) has confirmed that they reduce the width of the palpebral fissure. IVY (1972) had equally good results, but less side effects, with a 5% solution. However, the effect is not very marked. Moreover, guanethidine eyedrops have no effect on proptosis. In his careful study, KOUTRAS (1970) found no evidence of any beneficial effect on palpebral fissure or exophthalmos when prednisolone eyedrops were used or metraonidazole was given per os. We do not recommend local injection of corticosteroids or other agents, although GARBER (1966) has reported good results.

Treatment of thyroid hyperfunction must be carefully supervised in order to avoid iatrogenic hypothyroidism. We generally prefer to give radioiodine since gradual attainment of euthyroidism renders supervision easier. Some authors give radioiodine in small repeated doses. In the majority of cases the ophthalmopathy remains stationary after radioiodine or sometimes improves with a latency of several months.

Prednisone benefits many cases of severe ophthalmopathy (WERNER, 1966), but we usually reserve this treatment for emergency cases where vision is threatened by severe keratitis or optic nerve atrophy, or when there is a rapid progression of extra-ocular muscle paralysis. High doses of 50–100 mg/day are necessary for

such patients, and WERNER (1966) recommends even higher doses (up to 140 mg) in refractory cases. The prednisone should be tapered to the lowest effective dose after 2 to 3 weeks. It should be given for at least 4 weeks, but no guidelines exist as to whether it should then be stopped or whether it should be continued for several months. The mode of action of prednisone is not known. GREEN (1963) and PLIMSTONE (1964) have reported a decrease in LATS levels during prednisone medication, but since the role of LATS in the eye disease is controversial, this is no satisfactory explanation for the prednisone effect. In some cases prednisone brings about a dramatic improvement of the ophthalmopathy.

Immunosuppressive therapy with azathioprim may lower LATS levels. It has shown benefical effects on the eye changes in one study (WERNER, 1967a) and no effect in others (HADDAD, 1967; BURROW, 1970).

X-ray treatment directed toward the orbital space with antiinflammatory doses of 800 – 1000 rad has often been recommended, and HORST (1960) was able to completely eliminate the use of operative decompression procedures by this means in a large series of cases, but others have not had equal success. More recently DONALDSON (1973) has had very good results with higher radiation doses of about 2000 rad applied by supervoltage. It remains to be seen whether this is tolerated without any late adverse effects.

Hypophysectomy and pituitary irradiation have been popular for some time and the term "thyrohypophyseal syndrome" was coined under the assumption that endocrine ophthalmopathy was caused by a pituitary factor (LAMBERG, 1957). Most centers have abondoned this form of treatment due to lack of efficacy.

Total thyroidectomy and radioablation of the thyroid gland are advocated by CATZ (1965) and by BAUER (1966). This treatment is based on the attractive hypothesis that infiltrative ophthalmopathy is an autoimmune process which is kept active by the presence of antigens in the thyroid gland. Other authors (WERNER, 1967b; PEQUEQNAT, 1967; VOLPÉ, 1969) have not, however, been able to produce the same good results as Bauer. This form of treatment therefore remains controversial.

When control of thyroid hyperfunction and prednisone therapy have failed to arrest the progression of malignant ophthalmopathy, and in particular when vision deteriorates, *surgical decompression* must be performed. The indications for decompression are progressing decrease of vision or visual field defects and severe kreatitis, both resistant to medical management. This treatment, formerly considered a heroic last-resort measure, should not be delayed too long, since modern surgical techniques have greatly improved the results. Even with the availability of modern techniques, operations should *never* be undertaken on cosmetic indications. Many authorities consider surgery the only really effective form of treatment of severe proptosis. The choice of operative technique depends on the surgeon available. Neurosurgeons will perform the Naffziger (1932) operation (RILEY, 1972). An extradural approach is used to perform a transfrontal craniotomy and the ceiling of the orbital cavity is then opened. The bone is removed and a longitudinal incision is made in the fascia to allow expansion of the compressed orbital content.

We agree that a transantral approach (OGURA, 1962) with removal of the floor and lateral walls of the orbita is now probably the best surgical approach (*Editorial, Brit.med.J.* 1972; DESANTO, 1972). This procedure avoids a craniotomy and in our limited experience it has provided dramatic relief.

In a few selected cases with diplopia stationary over two years, corrective surgery on the extraocular muscles is possible. Alternatively, prism spectacles sometimes afford some relief.

3. Toxic Nodular Goiter (Toxic Adenoma, Plummer's Disease)

Graves' disease is characterized by diffuse hyperfunction of all the thyroid tissue and by certain almost specific extrathyroidal (ocular and dermal) signs.

In contrast, in toxic nodular goiter the hyperfunction remains localized to parts (nodules) of the thyroid gland and there are no extrathyroidal signs except those due to thyroid hormone excess.

Toxic nodular goiter should be strictly differentiated from Graves' disease occurring in a formerly euthyroid multinodular goiter (p. 178). Before scintiscans combined with T_3 suppression or TSH stimulation tests were available, the differentiation between these two forms of hyperthyroidism was often a matter of guesswork. Although the presence of ophthalmopathy is virtual proof of Graves' disease, its absence does not establish the diagnosis of toxic nodular goiter.

PLUMMER (1913) was the first to suggest that thyrotoxicosis could be produced by hyperfunction of thyroid nodules, but clinical differentiation between toxic nodular goiter and Graves' disease subsequently proved very difficult. The autoradiographic studies of COPE (1947) definitely established toxic nodular goiter

as an entitity, and HORST (1960, 1967), by improving on the scintiscanning technique drew attention to its relatively high frequency; he has accumulated experience with more than 300 cases within a relatively short time.

a) Incidence

In the large thyroid centers of the U.S.A. only a few cases of toxic adenoma are diagnosed each year, but an older study from the Presbyterian Hospital in New York City reports that of 2431 hyperthyroid patients operated upon between 1924 and 1944, 753 suffered from toxic nodular goiter (WERNER, 1971). Even if one admits that a considerable proportion of these may have been cases of Graves' disease in nodular goiters, the incidence of toxic nodular goiter is still appreciable. HALL (1970), without giving his source of information, cites that in the United Kingdom 5% of all cases of hyperthyroidism are due to toxic adenoma. A recent English study has estimated that toxic adenoma accounts for about 8% of all cases of hyperthyroidism (FERRIMAN, 1972). HORST (1960, 1965, 1967) found toxic adenoma in 30% of patients with hyperthyroidism in both Hamburg and Zurich. Hamburg is entirely free of endemic iodine-deficiency goiter, while Zurich still suffers from some mild iodine deficiency with a relatively high incidence of euthyroid goiter. In the Salzburg (Austria) area, where goiter is endemic, toxic adenomas account for 46% of all cases of hyperthyroidism (POHL, 1973). The reason for the discrepancy in incidence between continental Europe and the United Kingdom and U.S.A. is not readily apparent. Iodine-deficiency goiter may predispose to the development of toxic adenoma, as suggested by the high incidence in Salzburg. However, iodine deficiency cannot explain the high incidence in Hamburg. The discrepancy may be due partly to different scintiscanning techniques (p. 208). The female : male ratio in HORST's (1967) series was 6 : 1 and in POHL's (1973) series 8 : 1. Toxic adenoma does occur as early as the third decade of life, although this is rare. The peak incidence is between 60 and 70. In Zurich, 7% of toxic adenomas were found in recurrent goiter after previous thyroidectomy for euthyroid goiter (HORST, 1967).

b) Pathologic Anatomy and Pathophysiology

HORST (1967) found uninodular goiter in 75% and multinodular goiter in 25% of his cases. Whether toxic adenomas should be considered as true neoplasms or as part of a nodular goiter is a question of semantics, since it is always quite difficult to differentiate nodules of a multinodular goiter from true follicular adenomas on pathological grounds (p. 228).

Radioiodine investigations suggest that toxic adenomas are not under TSH control. If triiodothyronine is given to the patient to completely suppress TSH secretion, the adenoma will still take up radioiodine, while the surrounding thyroid tissue becomes inactive (Figs. 22, 23 b). When the thyroid hormone production of the adenoma causes only a borderline hyperthyroid state, the TSH secretion is not entirely suppressed and the surrounding thyroid tissue is still visible in the scintiscan (Fig. 23 a, so-called "compensated" toxic adenoma of HORST (1960)). In compensated toxic adenomas clinical signs

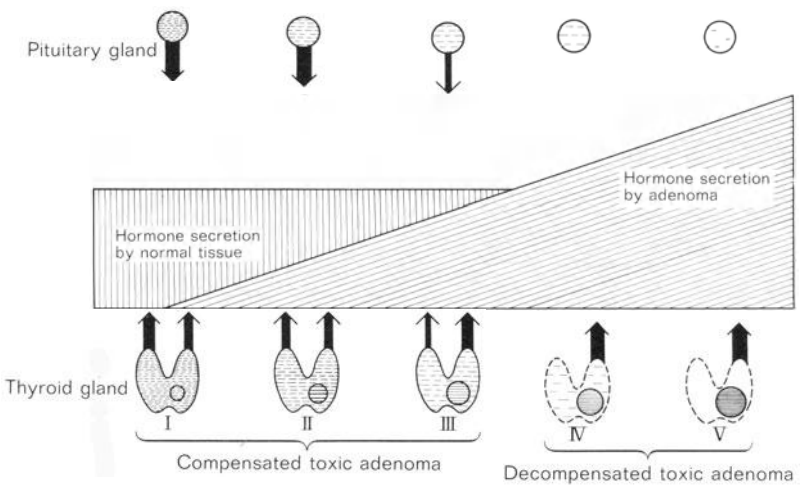


Fig. 22. Development of a warm nodule into a toxic adenoma of increasing size and with increasing thyroid hormone output. As the thyroid hormone hypersecretion progresses the TSH release successively declines and the remaining thyroid tissue becomes inactive. (BAY, 1965)

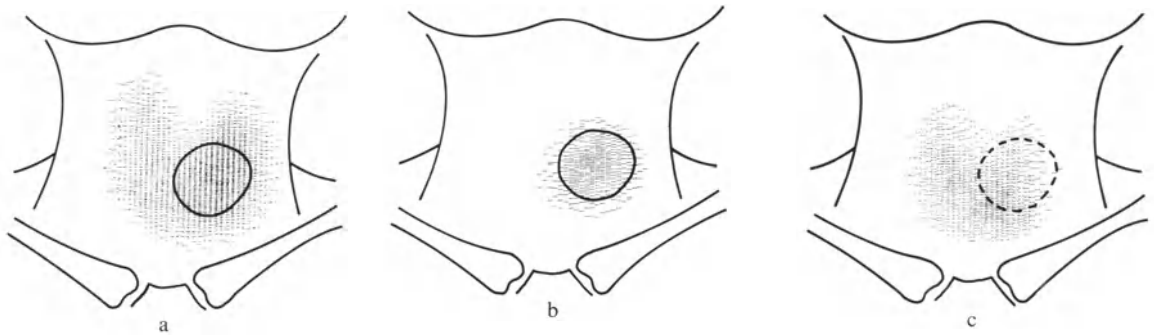


Fig. 23a–c. So-called "compensated" toxic adenoma. a) Scintiscan shows that the palpable nodule takes up more radioiodine than the surrounding tissue. b) Repeated scintiscan after short triiodothyronine treatment. Only the nodule takes up ^{131}I. c) Repeat scintiscan 3 months after radioiodine treatment. The nodule is "cold". (Courtesy of Dr. H. RÖSLER, Inselspital, Bern)

are often discrete and thyroid function tests are within normal limits. However KARLBERG (1973) has shown that in these patients TSH secretion after injection of TRH (p. 242) is impaired, which constitutes the most sensitive evidence of thyroid hormone oversecretion. If the adenoma causes frank hyperthyroidism, endogenous TSH secretion is entirely suppressed and the surrounding glandular tissue does not take up radioiodine. Injection of bovine TSH for several days will activate the remaining thyroid tissue which then becomes visible on the second scintiscan (Fig. 24, so-called "decompensated" toxic adenoma of HORST (1960)). Decompensated toxic adenomas are usually larger than compensated adenomas. Evolution from the compensated to the decompensated stage has been observed in a few cases. After inadequate radioiodine treatment a decompensated toxic adenoma may become compensated again.

The accelerated iodine turnover of toxic adenomas causes the phenomenon of "*emptying*". If the scintiscan is repeated one or two weeks after the diagnostic radioiodine dose, the adenoma appears "cold" (i.e. to contain no radioactivity) while the remaining tissue does still contain appreciable radioactivity.

The fact that functioning adenomas must have a certain size (usually 2 cm in diameter) to produce toxic symptoms suggests that the tissue mass probably plays a critical role in the thyroid hormone oversecretion. Recently, ERMANS (1971) has drawn attention to another critical factor. For several months he gave 500 µg of iodine daily to 4 euthyroid patients with warm nodules. In all 4 patients the serum hormone level rose into the thyrotoxic range, and 3 patients became clinically hyperthyroid. It thus appears that the nodules have lost their capacity to adapt to moderate iodine loads. They pick up any iodine presented to them and transform it into thyroid hormone.

The biochemical basis of the escape of toxic adenomas from TSH control is not known. BURKE (1972) and LARSEN (1973) examined toxic nodules in vitro. Surprisingly the nodules responded to TSH added *in vitro*, but the response was exaggerated. This suggested TSH hypersensitivity rather than autonomy.

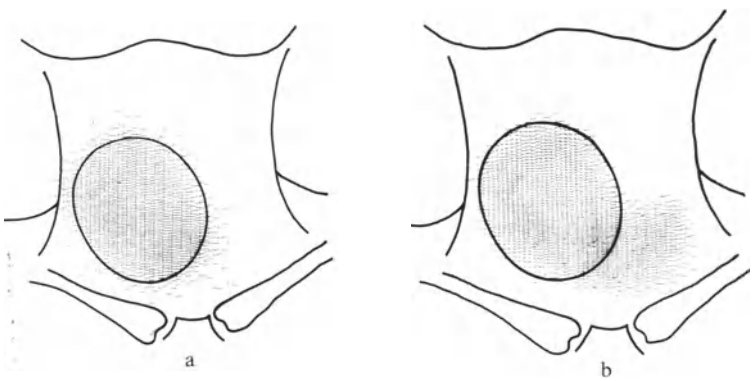


Fig. 24a and b. Large "decompensated" toxic adenoma. a) Initial scintiscan. There is no radioiodine uptake in the left lobe. b) Repeat scintiscan after a course of TSH injections. The left lobe is now clearly visible. (Courtesy of Dr. H. RÖSLER, Inselspital, Bern)

c) *Clinical Features of Toxic Nodular Goiter*

While in typical Graves' disease the patient presents with severe weight loss, nervousness, overactivity, diffuse goiter and exophthalmos, the characteristic picture of toxic adenoma is much more subtle: the classic presentation is that of an elderly woman with only minimal weight loss, a uninodular goiter and with some "functional" signs unusual for her age, such as warm moist hands, a stare and tachycardia (Table 12).

Table 12. Comparison of clinical features in toxic nodular goiter and Graves' disease

	Toxic nodular goiter (HORST, 1967) (%)	Graves' disease	
		VIGIER (1964) (%)	WAYNE (%)
Irritability, nervousness	72	90	59
Palpitation, tachycardia	56	95	75
Thermophobia, perspiration	41	70	68
Weight loss	25	95	52
Diarrhea	10	50	8

The onset of the disease is insidious and cannot be accurately established in most cases, in contrast to the onset in Graves' disease. An average of 5 years elapses from the beginning of the first symptoms to when the patient seeks medical advice. In one study, in 27% of "compensated" adenomas and 7% of "decompensated" adenomas the diagnosis was made incidentally during the diagnostic work-up for a supposedly euthyroid goiter (HORST, 1967).

The most frequent complaint is increased irritability (Table 12). Palpitations, tachycardia and heat intolerance follow on the list of clinical features. Weight loss is present in only 25% of cases. Very often the clinical picture is oligosymptomatic and the patient consults his doctor for nonspecific cardiac symptoms. Paroxysmal or chronic auricular fibrillation is frequent.

Occasionally mental changes with depression or loss of libido predominate. With the chronic slow onset the patient is often well adapted to his disease and has no subjective symptoms. In a few cases however, the picture of full-blown hyperthyroidism develops. Myopathy is present in about 10% of patients. Endocrine ophthalmopathy with exophthalmos has never been observed with toxic adenoma in either the Hamburg series or the Zurich series. In contrast, however, lid lag and a stare do occur.

Although the thyroid hormone secretion of compensated toxic adenomas is by definition not enough to entirely suppress the endogenous TSH release, it can still produce mild clinical signs and symptoms, in our experience related to the cardiovascular system in most cases. The reason for this is not readily apparent, and more research on the serum concentrations of thyroxine and triiodothyronine (in particular the free hormone fraction) in this form of toxic nodular goiter is certainly necessary.

Table 13. Synopsis of clinical features and treatment of toxic nodular goiter compared to Graves' disease (HORST, 1967)

	Graves' disease	Toxic nodular goiter
% of all cases of thyrotoxicosis (Zurich)	70	30
History	Rapid onset in many cases	Insidious onset, oligosymptomatic
Clinical findings	Diffuse goiter (occasionally multinodular goiter), all degrees of severity of hyperthyroidism. Ophthalmopathy in about 50% of cases	Uninodular goiter in most cases, few symptoms and signs of mild grade. No ophthalmopathy
Diagnosis	Can be established on clinical grounds in most cases. Serum thyroxine elevated. LATS positive in 20–50%	Clinical diagnosis difficult. Serum thyroxine and basal metabolic rate often borderline elevated. LATS always negative
Treatment	Radioiodine: 7000 to 10000 rad Subtotal thyroidectomy, Thionamide drugs	Radioiodine: 20000 to 30000 rad under T_3 protection. Selective operative resection of adenoma
Risks of treatment	Radioiodine: Hypothyroidism 25–70% Surgery: Hypothyroidism 6–50% Hypoparathyroidism Recurrent nerve palsy Drugs: Toxicity in ca. 5%	Radioiodine and operation: practically nil
Relapse rate	Radioiodine: 10% Surgery: 6–25% Drugs: 50%	Radioiodine and surgery: practically nil

d) Course of Toxic Nodular Goiter

Unlike Graves' disease, toxic adenomas have no tendency to spontaneous remission. With time the adenomas may grow, secrete more hormone and produce more symptoms. SILVERSTEIN (1967) has followed 9 cases over 2 to 7 years and has found that the course is variable, but generally benign. The course may be influenced by fluctuations in dietary iodine supply which in turn cause variations in thyroid hormone output (p. 206). Toxic adenomas of long standing may show degenerative cystic and necrotic changes in the center, which have been attributed to increased tissue pressure (BAY, 1965). Enough functioning tissue usually remains at the periphery of the nodule to maintain thyroid hormone oversecretion. Spontaneous cures are rare.

e) Diagnosis and Laboratory Tests

Uninodular goiter with discrete signs of hyperfunction and without ophthalmopathy arouses a strong suspicion of toxic adenoma, which in HORST'S (1967) series was confirmed in 80% of such cases. The PBI (representing mostly thyroxine) is usually slightly elevated (BAY, 1965). STERLING (1970) published several cases with isolated oversecretion of triiodothyronine. The basal metabolic rate does often not exceed the normal limits. In a series of 53 cases it was +18% in "decompensated" and +8% in "compensated" toxic adenomas. The ankle reflex time is slightly shortened.

The radioiodine uptake is within normal limits in many cases. In HORST'S (1967) series the mean uptake was 33% at 2 hours and 50% at 48 hours (the mean normal value in Zurich being 19% and 45%). In a parallel series of patients with Graves' disease Horst found uptakes of 53 and 62% at 2 and 48 hours respectively. The PB ^{131}I at 48 hours is grossly elevated to a mean of 1.26%/liter (mean normal 0.08%/liter).

The diagnosis is established by tracer studies combined with a scintiscan (Figs. 23, 24). Repeat scintiscans are performed after triiodothyronine suppression in cases of "compensated" adenomas or after TSH stimulation in cases of "decompensated" adenomas. Most commercial scanners have a relatively high cutoff (amount of radioactivity recorded as zero on the printout) with a superlinear (highcontrast) printout for radioactivity exceeding the cut-off point. This results in "sharp" scintiscans with no background. However, details, e.g. a warm nodule within the gland, may be overlooked with this technique and HORST (1960) therefore recommends a low cut-off with a linear low-contrast print-out. Only extensive studies of this type allow definite differentiation between true toxic adenoma and Graves' disease in a multinodular goiter. The differentiation has important therapeutic consequences.

f) Treatment of Toxic Nodular Goiter

Since, unlike Graves' disease, toxic adenomas do not remit spontaneously, drug therapy would have to be lifelong. Thionamide drugs are of course effective in inhibiting the thyroid hormone biosynthesis of toxic adenomas. In selected cases they may be given as a temporary measure until a definite form of therapy is applied. The available modes of curative treatment are radioiodine and surgery. Both modes of treatment differ from those applied in Graves' disease. An important point is the much better prognosis with respect to posttherapeutic hypothyroidism.

The treatment of choice in our view is *radioiodine* with ^{131}I given under triiodothyronine suppression to protect the normal thyroid tissue. The dose must be high, namely 20000 to 30000 rad, as compared to 10000 rad in Graves' disease. Lower doses are ineffective or only transform a "decompensated" into a "compensated" adenoma. The average dose is 30000 rep/adenoma. The TSH effect following the TSH stimulation test must have worn off before the therapeutic radioiodine dose is given. Protective triiodothyronine therapy is indicated in all cases. Although in "decompensated" adenoma the normal thyroid tissue does not accumulate radioiodine initially, it will soon start to do so, when the adenoma slows down its hormone secretion in response to treatment. Triiodothyronine is started on the day before treatment at a dose of 20 µg every 6 hours, and is continued for 10 days. If cardiovascular symptoms represent a contraindication to triiodothyronine treatment, reaccumulation of ^{131}I into the normal tissue can be blocked by perchlorate started 48 hours after the radioiodine.

With correct treatment the success rate approaches 100%. Posttherapeutic hypothyroidism is extremely rare. Recurrences have been seen in few cases. The whole-body dose is usually lower than in the treatment of Graves' disease, despite the fact that the radiation dose to the adenoma tissue is three times greater. Proof of cure are the disappearance of clinical signs and symptoms and the absence of radioiodine accumulation in a follow-up scintiscan in the region of the adenoma, while uptake in the surrounding parenchyma is normal. The toxic adenoma must have been transformed into a "cold" region.

Surgery is indicated in the following circumstances: 1. When there are "cold" areas (i.e. areas with no ^{131}I uptake) in the nodules or other signs arousing the suspicion of malignancy. Several cases of malignant degeneration of toxic adenomas or of cold malignant nodules coexisting with benign toxic adenoma have been reported (GUINET, 1971). This is the main reason why French authors prefer surgical treatment for toxic adenoma. 2. When the radioiodine turnover in the adenoma is such that the necessary ^{131}I dose is so high as to produce a whole-body dose above permissible limits. 3. In adolescents and children.

The operation consists of selective removal of the adenoma with conservation of all the normal thyroid tissue. The thyroidal arteries need not be ligated and the risks of postoperative myxedema, hypoparathyroidism or recurrent nerve palsy are negligible (ZUKSCHWERDT, 1963). The question as to whether preoperative medical control of the hyperthyroidism is as necessary in toxic adenoma as in Graves' disease is not yet settled. During the selective removal of the adenoma the blood vessels can be carefully ligated, and the risk of large amounts of hormone being released is low. According to experience in Hamburg and Paris, 60 toxic adenomas were resected without prior drug pretreatment with no serious complications. FUCHSIG (1968) observed one case of thyroid crisis in 100 operations for toxic adenoma.

4. Rare Causes of Hyperthyroidism

a) Hydatidiform Mole

In normal pregnancy thyroidal radioiodine uptake and total serum thyroxine concentration are both increased. The former is still largely unexplained, while the latter is due to raised thyroxine-binding globulin (TBG) level. Thus, the free thyroxine remains normal.

In molar pregnancy similar changes occur, but they are more pronounced. Interestingly enough, most patients are clinically euthyroid, even though the free thyroxine concentration is increased (ODELL, 1963; GALTON, 1971), but cases with severe clinical hyperthyroidism have also been reported (HERSHMAN, 1971).

The observation that patients suffering from hydatidiform mole or chorionic carcinoma are sometimes hyperthyroid and that the thyroid hyperfunction is cured by removal of the tumor has led to a search for thyrotropic factors in chorionic tissue (BURGER, 1967). Two unrelated substances have been characterized. One can be extracted from molar tumors (and serum of patients). Its onset of action in the mouse bioassay is between that of TSH and that of LATS. It is immunologically different from TSH and it is not an immunoglobulin (HERSHMAN, 1970; GALTON, 1971). The other substance occurs in normal placenta and has been called chorionic thyrotropin. It has the same molecular weight as TSH and cross-reacts with TSH antibodies (HERSHMAN, 1970). Hyperthyroidism with a circulating TSH-like substance has also been seen in a patient with a testicular embryonal carcinoma (STEIGBIGEL, 1964).

b) Thyroid Carcinoma

Thyroid carcinomas very rarely produce thyroid hormone. Nonetheless, several cases which have caused clinical hyperthyroidism have been reported (HUNT, 1960; STUDER, 1961; FEDERMAN, 1964; GHOSE, 1971).

c) TSH-Secreting Pituitary Tumors

As a rule TSH secretion by the pituitary is suppressed in thyrotoxicosis. With the availability of TSH radioimmunoassay a few cases of TSH-secreting pituitary tumors have been detected. In one case the adenoma produced concomitant acromegaly (HAMILTON, 1970 and 1972). Two cases reported by FAGLIA (1972) suffered from isolated TSH oversecretion. The case studied by MORNEX (1972) suffered from hypopituitarism due to the adenoma. One of EMERSON's cases (1972) was attributed to oversecretion of TRH.

d) Iodide-Induced Thyrotoxicosis ("Jod-Basedow")

The pioneers in the thyroid field, COINDET, RILLIET, KOCHER (1910) and DEQUERVAIN observed many cases of iodide-induced thyrotoxicosis. Later this form of thyrotoxicosis was somehow forgotten, and many doubted whether it really existed. STANBURY (1954) recalled attention to the condition when one of his endemic goiter patients in Argentina developed severe hyperthyroidism under an iodine supplement of 1.5 mg per day.

ADAMS (1968) implicated LATS in the pathogenesis, but we think that the German term "Jod-Basedow" is misleading. The disorder is most probably not due to Graves' disease. KOCHER (1910) never observed exophthalmos in his cases. The pathophysiologic basis are probably functioning autonomous thyroid nodules which have escaped control by TSH. These nodules cannot produce excess thyroid hormone as long as the dietary iodine supply

is inadequate or in the low normal range. As soon as the dietary iodine is raised, the nodules avidly pick it up and synthesize excess hormone. The amount of dietary iodine producing hyperthyroidism is generally thought to be 5 mg/day or more. However, ERMANS (1971) has recently produced iodide-induced hyperthyroidism with as little as 0.5 mg/day. CONNOLLY (1970) observed a sharp rise in the incidence of thyrotoxicosis in Tasmania where goiter was endemic after the bread had been iodized to supply about 150 μg of iodine per day, and similar observations are recorded in the American literature of the 'twenties (see review by STANBURY, 1954).

In our view, the occurrence of iodide-induced thyrotoxicosis is no argument against the use of iodine in the prevention of endemic goiter, but it suggests that it might be wise to supplement low doses during the first decades. KOCHER (1910) himself recommended small doses of iodide for the prevention of iodide thyrotoxicosis.

The belief that iodide-induced thyrotoxicosis only develops in areas of iodine deficiency has been badly shaken by a recent report by VAGENAKIS (1972). He gave large doses of iodide to 8 patients with euthyroid goiter who resided in Boston, an area where the iodine supply is known to be more than adequate. Four patients developed hyperthyroidism, which was severe in two cases. The hyperthyroidism worsened initially when the iodide was withdrawn.

e) Factitious Thyrotoxicosis and Struma Ovarii

Voluntary excess thyroid hormone intake is rare. ROSE (1969) published three cases, all of them women. The patients persistently denied thyroid hormone intake, but this was demonstrated beyond all doubt by a high serum PBI with a very low thyroidal radioiodine uptake. All three patients were severely neurotic. KIRKEBY (1972) reported a patient who attempted suicide by ingesting 40 mg of thyroxine. He survived the intoxication.

Struma ovarii is an extremely rare cause of hyperthyroidism (BROWN, 1973).

G. Endemic Thyroid Disease

Two closely related clinical syndromes are grouped under the heading of *endemic thyroid disease*: endemic cretinism and endemic euthyroid goiter. The two are related by their pathogenesis, in which dietary *iodine deficiency* plays a dominant, although not an exclusive role. Endemic goiter can occur in areas where there is no endemic cretinism. It is a manifestation of *moderate* iodine deficiency. Endemic cretinism is seen only in areas where there is concomitant endemic goiter. It is a manifestation of *severe* iodine deficiency. Goiter is arbitrarily defined as endemic when 20% or more of children between the ages of 12 and 14 years have a palpable thyroid gland.

The iodine content of food and drinking water shows wide geographic variations. Inland regions, and especially mountainous regions, are particularly iodine-deficient. The areas with iodine deficiency are largely identical with areas of previous glaciation (MERKE, 1965). It is suggested that the water melting from the glaciers has washed out the iodine from the soil covered by ice. In the Canton of Valais (Switzerland), for example, endemic thyroid disease was much more prevalent at the bottom of the valley than in the villages situated higher up on the slopes (MERKE, 1967). The latter areas had not been glaciated. Geological surveys in Greece have shown that "goitrous" villages are often situated on noncalcareous rock and obtain their water from superficial polluted wells. The surrounding soil binds iodine firmly (MALAMOS, 1971). Maritime regions are generally iodine-rich.

The classic endemic regions were the Alps, the Pyrenees, and the Himalayas. In the past decades foci of severe endemicity have been found in the South American Andes, in the Congo, in New Guinea and in Thailand. Goiter was endemic in the region of the Great Lakes in the U.S.A., but cretinism did not occur. Cretinism has been or is still found in the other regions mentioned above. Endemic thyroid disease constitutes a major public health problem in many countries. It has therefore received the attention of large organizations and a number of good reviews are available (WHO, 1960; KÖNIG, 1968; RICCABONA, 1972). The latest findings have been collected in a symposium edited by STANBURY (1972).

1. Endemic Euthyroid Goiter

a) Etiology

As outlined above, dietary iodine deficiency is the major etiologic factor in endemic goiter. The renal clearance for iodide is fixed (30–40 ml/min) and the plasma iodide level therefore depends solely on the dietary iodine intake.*

* The fact that the iodide clearance of the thyroid gland is highly variable does not modify this statement. In a steady state the thyroid returns to the plasma the same amount of iodine, in the form of hormones, as it takes up. The hormones are deiodinated peripherally to iodide.

With a plasma iodide concentration of 0.1 μg/100 ml the thyroid function remains normal and no goiter develops. At this serum concentration 50 μg of iodide are lost each day in the urine when the clearance is 35 ml/min. Another 20 μg of iodine lost in the stools will give 70 μg/day, the minimum amount of dietary iodine necessary for normal thyroid function. Most authorities add a safety factor for individual variations of renal clearance and for periods of increased demand (pregnancy), and recommend an iodine intake 200 μg/day (WAYNE, 1964). Populations with average daily intakes of below 40 μg usually have at least some degree of endemic goiter.

The iodine-deficiency theory is based on several facts: 1 In endemic regions the iodine content of the drinking water (FELLENBERG, 1933) and the urinary iodine excretion (which is a good measure of the intake) are both low (LAMBERG, 1962); 2. Iodine supplements, whether in the form of iodine added to table salt or of iodized oil injections, have effectively eradicated endemic goiter in some countries; 3. Iodine deficiency produces goiter in experimental animals.

Several facts seem at first glance not to be compatible with the iodine-deficiency theory: 1. Not all people in an endemic area develop goiter. A possible explanation for this is that there is wide interindividual variation in the iodine intake. In Glasgow, for example, values from 40–1 000 μg/day were measured (WAYNE, 1964). Moreover, the renal iodide clearance may be elevated in some people who develop goiter (CASSANO, 1961). Partial enzyme defects which are not expressed in iodine abundance may become manifest in iodine deficiency. Thus, slightly pathologic perchlorate discharge tests have been found in goitrous subjects. BECKERS (1962) found a decreased proteolytic activity in the thyroid glands of goitrous individuals; 2. In regions of mild to moderate endemicity, goiter is more prevalent in women than in men. Sex-linked alterations in thyroid metabolism or some influence of female sex hormones may be proposed as an explanation, but there is no reliable proof of this.

Goiter in an endemic area often occurs in families. This, however, is no reliable argument for an hereditary etiology of endemic goiter, since familial occurrence may be explained by common dietary habits in all members of a family. The minor role of hereditary factors is documented by observations that people immigrating into an endemic area develop goiter, while in people leaving the endemic area the goiter sometimes regresses.

The opponents of the iodine-deficiency theory also cite the following good arguments: 1. Closely related populations with an identically low iodine intake have widely diverging goiter prevalence rates. A good example is Idjwi Island in Zaire (formerly Republic of Congo). In the southwest of the island, the incidence of goiter is much lower than in the north, although both populations are exposed to a similar degree of severe iodine deficiency (DELANGE, 1968); 2. In several surveys the prevalence of goiter was found to correlate poorly with the iodine content of the drinking water (DAY, 1972). Correlation of goiter with water hardness, fluoride content, or in some studies pollution of the drinking water (GAITAN, 1969) was better; 3. COSTA (1963) has observed epidemics of goiter unrelated to iodine deficiency in Italy from 1940 to 1948; 4. Some populations seem to adapt to severe iodine deficiency without goiter formation or other signs, except for an elevated ^{131}I uptake (ROCHE, 1959; LAMBERG, 1962; CHOUFOER, 1963).

One explanation for all these discrepancies is that in addition to iodine deficiency, populations may be exposed to varying amounts of goitrogenic substances. In Idjwi Island and other parts of Africa cassava is an important foodstuff. The roots of this plant contain a substance (cyanogenic glucoside) which releases cyanide, which is detoxified within the body to thiocyanate (CNS^-) and this acts as a goitrogen by inhibiting thyroidal iodide transport (EKPECHI, 1965). Goitrin (vinyl-thiooxazolidon) is another goitrogenic substance found in food. It inhibits iodination of thyroglobulin, as do the thionamide drugs. It is present as progoitrin in several plants such as cabbage, turnips and rape, and is liberated from its precursor by vegetable or bacterial enzymes. Plants containing progoitrin are rarely ingested by man in amounts sufficient to cause goiter. However, the milk of cows fed on certain plants of the Brassica family contains sufficient goitrin to cause goiter in man (CLEMENTS, 1960).

Other weak goitrogenic substances are nitrate (NO_3^+) and calcium ion (Ca^{++}). Lithium is a relatively potent goitrogen (SCHOU, 1968) (p. 154). Some otherwise healthy people cannot perceive the bitter taste of the goitrogen phenylthiocarbamide. It has been suggested that this hereditary variant predisposes them to the intake of natural goitrogens and therefore to goiter formation (KITCHIN, 1960).

The currently prevalent, although not universally accepted, opinion on the etiology of endemic goiter is that iodine deficiency usually has a dominant role. Naturally occurring goitrogens are additional factors which may greatly modify the quantitative effects of iodine defi-

ciency. Only very few endemic regions are known where the iodine supply is adequate.

We must add that iodine excess can also cause goiter (p. 154). In Hokkaido (Japan), where the residents of the coastal area consume 10000 to 20000 µg of iodine/day in algae, iodine-induced goiter is endemic (so-called coast goiter, SUZUKI, 1965; cf. p. 154).

b) Pathogenesis and Pathophysiology

The mechanisms by which iodine deficiency produces goiter have been extensively studied and reviewed by STUDER (1965, 1968, 1974). The normal human thyroid gland contains appreciable stores of iodine which would be sufficient to provide the body with hormone for several months. However, STUDER's studies (1974) have shown that thyroid hormone secretion falls rapidly when iodine input into the gland is prevented in some way. The basis of this rapid adaptation is the subdivision of the thyroidal organic iodine pool into several compartments. Fast-turnover compartments contribute a large part of the daily hormone secretion. As soon as these compartments are depleted, hormone secretion diminishes, serum hormone level falls slightly and TSH secretion is triggered long before the gland as a whole is significantly iodine-depleted. TSH in turn stimulates many processes in the thyroid gland, the end result of which is a massive increase in the thyroidal iodide clearance. Instead of taking up 30% of a radioiodine dose, as in iodine-replete areas, the gland now takes up 80% or 90% of the dose. Correspondingly less radioiodine is lost in the urine. Only recently has it been possible to measure an increase in serum TSH levels in goitrous subjects from areas where the condition is endemic (BUTTFIELD, 1966; ADAMS, 1968; COBLE, 1970). TSH is probably the main factor in the adaptation to iodine deficiency, but so-called thyroidal autoregulation, which is difficult to demonstrate in man, may also be involved. The result of these adaptive mechanisms is that in general the *total* iodine content of the whole gland is within normal limits. The iodine content/g thyroid weight, however, is greatly diminished, and the degree of iodination of thyroglobulin (µg iodine/mg thyroglobulin) is equally low. This degree of iodination of thyroglobulin is a critical factor in hormone synthesis. At the low degrees found in goiter most of the iodine is in the form of the early hormone precursor monoiodotyrosine. Little diiodotyrosine and even less hormone are formed (ERMANS, 1968). Thus the low degree of iodination of thyroglobulin initiates a vicious circle, since the little iodine available is inefficiently utilized. On hydrolysis of thyroglobulin a large amount of iodoamino acid precursors and little thyroid hormone are released. The precursors (or the iodide generated from them) may leave the gland and contribute to the increased non-hormonal iodine escape found in endemic goiter (ERMANS, 1963). The preponderance of monoiodotyrosine over diiodotyrosine is more favorable for the formation of triiodothyronine than for that of thyroxine. Increased triiodothyronine secretion has been found in iodine-deficient rats by STUDER (1965, 1968). More recently DELANGE (1972) has demonstrated serum triiodothyronine levels twice as high as normal in endemic goiter subjects, while the thyroxine level was half the normal value. Since triiodothyronine is much more active (per unit weight) this allows efficient and economic maintenance of a euthyroid state.

TSH stimulation in endemic areas produces typical radioiodine turnover data: a rapid and high thyroidal radioiodine uptake and a relatively rapid release of labeled hormone with a high PB ^{131}I. ERMANS (1963) found this was not so in an appreciable number of goitrous subjects. These so-called "slow secretors" took up radioiodine equally avidly but released it very slowly, and the PB ^{131}I was low normal. The reasons for the difference between fast and slow secretors are different iodine content and compartmentalization of iodine within the thyroid gland.

The cause of thyroid enlargement in iodine deficiency is the continuous TSH stimulation. STUDER (1965, 1968, 1974) has shown that hypophysectomized rats do not develop goiters when exposed to iodine deficiency. Neonatal goiter (weight > 3 g) is not uncommon in areas where the condition is endemic. In most cases (except in very severe iodine deficiency) goiter develops during childhood. In boys the goiter sometimes regresses after age 12 or 14. In girls the goiter increases in size until age 17 or 18 and then remains stationary. Pregnancy and lactation bring about further increases. The enlargement of the gland is at first *diffuse*, and later the diffuse hyperplasia progressively gives way to multinodular hyperplasia. The nodules probably arise from so-called lobules, groups of 20 to 40 follicles bound together by connective tissue. Why some lobules become hypertrophic and others atrophic is not clear. TAYLOR (1952) found out by autoradiography with ^{131}I that in multinodular goiter there was a marked heterogeneity of the ^{131}I fixation within follicles and nodules. Nodular goiter is notoriously difficult to produce in experimental animals.

c) *Incidence of Endemic Goiter*

Despite the spectacular success of iodine supplementation in certain countries, there are still an estimated 200 million people who suffer from endemic thyroid disease. The incidence of goiter in different areas varies from 90% to 0.4%. The data on endemic goiter incidence have been reviewed by KELLY (1960) and KÖNIG (1968).

In areas of severe iodine deficiency both sexes are equally affected with goiter. In areas of moderate or mild deficiency women predominate in a ratio of 6 : 1. The sex difference has never been satisfactorily explained.

d) *Pathologic Anatomy*

The anatomical picture in euthyroid goiter is dominated by polymorphism and heterogeneity. Diffuse enlargement or nodule formation may combine with colloid accumulation or parenchymatous hyperplasia to give diffuse or nodular colloid goiters, or diffuse or nodular parenchymatous goiters, or any combination within a single gland. A single nodule within an otherwise normal gland is called an adenoma. There is probably no basic difference between a nodule in a multinodular goiter and a single adenoma, but it is generally said that the structure of an adenoma is more uniform in histologic appearance and that it compresses the surrounding tissue. In multinodular hyperplasia the nodules are sometimes less well defined, but even experienced pathologists will have difficulty in differentiating adenomas unequivocally from nodules (cf. p. 228).

The microscopical picture in parenchymatous goiter is characterized by many very small follicles, in colloid goiter by huge follicles sometimes attaining the size of cysts. The cyst walls may form papillary infoldings. Solid epithelial structures (trabeculae) are seen in both types.

In addition, degenerative changes with hemorrhage and necroses may add to the polymorphism of the goiter.

e) *Clinical Features of Euthyroid Goiter*

In many patients, endemic (and sporadic, see p. 221) euthyroid goiter causes few symptoms, and it is often an incidental finding in a health survey or a physical examination performed for an unrelated disease. Sometimes the patient himself has noted swelling of the neck. Large nodular goiters may cause severe airway obstruction by pressure on the trachea.

For palpation of the goiter the patient should sit in a relaxed position with the head tilted slightly backward. He is asked to swallow while the physician bimanually palpates the neck either from behind or from in front of the patient. A glass of water should be at hand to allow repeated swallowing. The size, firmness, and shape of the gland are evaluated and the presence of any nodules is recorded. A simple classification of goiter size is used in most surveys of goiter prevalence (Table 14). For more refined purposes goiter size may be given as gland weight as estimated by palpation (by an experienced doctor) or by measurements from scintiscans (p. 196).

Table 14. Simple system for grading the size of euthyroid goiter. (Modified from: HUNZIKER, 1920; WESPI, 1942; PEREZ, 1960)

Grade 0:	Gland not palpable, not visible, less than 4 times normal size
Grade I:	Gland palpable, not usually visible, more than 4 times normal size
Grade II:	Gland palpable and easily visible
Grade III:	Very large goiter, easily visible at some distance

The neck circumference over the thyroid cartilage should also be recorded. It is of little value for estimating goiter size, but may allow monitoring of the effects of treatment in a given person. Vascular bruits or murmurs arouse a strong suspicion of thyroid hyperfunction, but they also occur occasionally in large euthyroid goiters.

The normal thyroid is not palpable, except for the isthmus, which may be palpated in thin people. By definition, goiter is present when the gland is easily palpable or visible. Slight pressure over large goiters during palpation may elicit stridor as a sign of tracheal compression. A chest X-ray should be obtained to diagnose any intrathoracic extension of a goiter. If it is not clear whether an upper mediastinal mass is a goiter, fluoroscopy may be helpful. In goiter the mass moves upwards on swallowing. Suspected airway obstruction may be documented by X-rays of the neck and trachea. Large intrathoracic goiters can cause recurrent nerve palsy and Horner's syndrome (this is a sign of malignancy in most but not all cases) or even upper venous congestion.

Goiter in the neonate often causes airway obstruction, which may be relieved by tilting the child's head backward. In neonates goiter regresses rapidly with iodine or thyroxine therapy.

f) *Laboratory Tests in Endemic Goiter*

As outlined on p. 212, the hallmark of iodine-deficiency goiter is a high thyroidal radioiodine

uptake combined with a euthyroid clinical status, as documented by normal ankle reflex time and basal metabolic rate. The serum thyroxine (or the PBI) may be low normal or sometimes frankly subnormal. Euthyroidism is maintained by a normal or elevated triiodothyronine concentration (p. 212). Occasional subjects are hypothyroid (BUTTFIELD, 1966).

g) Prevention of Endemic Goiter

MARINE (1920) was the first to demonstrate on a large scale that endemic goiter could be prevented by iodine supplementation. Before preventive measures are taken, iodine deficiency must of course be established as the cause of goiter in a given area. Good measures of iodine intake are the iodine content of the drinking water and of locally grown food, but the simplest measure of iodine intake is probably the urinary iodine excretion. In a steady state it is almost equal to the intake, since only small amounts (10–20 μg/day) are lost in the stools).

As discussed on p. 211, endemic goiter is present in areas where the average daily iodine intake is below 40 μg per day. In optimal conditions, allowing for a generous safety factor, the daily allowance should be 200 μg to ensure normal thyroid function in most people, but iodine prophylaxis has already given good results with a much lower supplementation.

As the result of a compromise between supporters and opponents of iodine supplementation, the table salt was supplemented with only 5 mg of KI/kg salt in Switzerland from 1923 onward. Although this raised the intake by only 38 μg/day, the results were spectacular. No more cretins were born and checks for deaf-mutism* in 8-year-old school children revealed a markedly reduced incidence 8 years after the introduction of iodized salt (WESPI, 1945). The incidence of goiter in school children fell from 50% to a few percent. By 1950, nodular goiters, which before iodine supplementation were common in school children, were found only in people over age 25. Military statistics are particularly valuable, since with universal military service every male Swiss is seen by a medical board at age 19. Recruits from areas with endemic goiter had a goiter incidence of almost 100%. After iodine supplementation it fell to 14%. Between 17 and 65 per 1000 men were found unfit for service because of goiter before iodine supplementation, as compared to 1 per 1000 after.

Iodine supplementation was raised to 10 mg KI/kg in parts of Switzerland in 1953, and in the whole country from 1962. Neonatal goiter was present in 30% of babies in the Canton of Aargau before iodine supplementation in 1951*; its incidence gradually fell to 6% within 4 years of the introduction of the lower (5 mg/kg) supplementation regimen, to disappear completely with the higher (10 mg/kg) regimen (WESPI, 1966). The sales of iodized salt have fallen in Switzerland, from 90% at its peak to 80% of all table salt currently sold, but the widespread availability of frozen fish and other sea food probably compensates for this decrease (MERKE, 1968). If iodination of salt is discontinued altogether, the amount of dietary iodine falls back to goitrogenic levels (PODOBA, 1972).

In the U.S.A., where there is no fear of "Jod-Basedow", the iodine supplementation in the salt is much higher, 20–200 mg/kg. In consequence the iodine intake is very generous, although wide regional variations still persist (ODDIE, 1970).

The KI of iodinated salt may be oxidized on storage to I_2, which is then lost by sublimation. In some areas the more stable potassium iodate is therefore preferred. In Tasmania flour is used as a vehicle and the iodine reaches the population mostly in the bread (CONOLLY, 1970).

Iodine supplementation represents a big technical problem in underdeveloped areas with rural self-sufficient economy. Salt is not used at all, or it is produced locally and the large-scale distribution of iodinated salt is virtually impossible. A useful alternative is the intramuscular injection of 2 ml (adult dose) of iodinated poppy-seed oil, which provides a depot of 950 mg of iodine. The results have so far proved satisfactory in many parts of the world (PRETELL, 1972), but since the injections have to be repeated every 2 to 5 years, the task is formidable, especially in countries where there is a shortage of medical personnel.

The results of iodine prophylaxis have largely supported MARINE'S assertion that "simple" goiter is one of the most easily avoided diseases. Despite this, the paradox remains that even advanced countries, such as France, Germany and Austria were very reluctant to introduce iodine supplementation, and very slow in doing so. The fear of iodide-induced thyrotoxicosis ("Jod-Basedow", see p. 209) played an important role in the heated arguments which preceded the introduction of iodized salt in Switzerland. In Tasmania CONOLLY (1970) observed a sharp

* Deaf-mutism can be used as a valid indicator of endemic cretinism, as discussed by QUERIDO (1972).

* The Cantons were free to decide whether and when to introduce iodized salt. Some, such as Canton Aargau, were quite late in doing so.

rise in the incidence of thyrotoxicosis after the iodine intake was raised by 150 µg per day by the iodination of bread. In more recent studies the importance of the phenomenon has been played down, and PRETELL (1972) observed only 1 case among 2000 Peruvian subjects injected with iodized oil. Others have found a much higher incidence of "Jod-Basedow", and its exact importance remains to be established by further studies. In any case, "Jod-Basedow" should be no argument against iodine prophylaxis, but its occurrence perhaps suggests that low doses should be given during the first decades of supplementation. It is unfortunate that no exact figures are available on the incidence of thyrotoxicosis during the years of introduction of iodized salt in Switzerland.

h) Treatment of Endemic Goiter

Most patients with endemic goiter are euthyroid and have few symptoms, and the question as to whether treatment is necessary at all is therefore justified. Basically, we agree with MEANS (1963) that "there seems little sense in carrying a grapegruit-sized tumor about in one's neck for a lifetime when it can easily be removed". However, this statement is subject to considerable qualification, and the decision of whether or not to treat an endemic goiter depends largely on the medical and surgical resources available. Obviously, surgical treatment of all goiters in an area where the condition is endemic is impossible in many underdeveloped countries, so that treatment has to be reserved for goiter causing severe pressure symptoms and airway obstruction.

Medical treatment can be tried first, but it is often ineffective in colloid nodular goiters. *Iodine* in a dose of 200 to 300 µg per day may be tried, e.g. 6 drops of a 0.1% KI solution ($\triangleq$ 230 µg I). In children the dose can be raised to 500 µg/day. Treatment should be discontinued after 3 months if there are no signs of regression.

L-thyroxine, 200 µg/day, is probably preferable to iodine. In younger subjects the goiter sometimes disappears rapidly, but in many cases, especially of long-standing goiter, the results are unsatisfactory. The dose may be increased cautiously to 400 µg/day. If there is no improvement after 3 months, drug treatment should be abandoned.

Surgery should be discussed in cases where medical treatment has been unsuccessful. The surgical procedure consists of subtotal, or sometimes total, intracapsular thyroidectomy, which must be followed by lifelong thyroxine therapy (see below).

Radioiodine has proved a valuable alternative to surgery in patients who refuse operation or in whom the operative risk is judged to be high due to concomitant cardiac or pulmonary disease (RÖSLER, 1965). The dose is 10000–12000 rad (on the average 40 mCi of ^{131}I). In cases of very large goiters weighing over 600 g, the ^{131}I is given in fractionated doses. When the radioiodine uptake is low ($<40\%$) or inhomogeneous, good results have been obtained by previous stimulation with TSH. On average the goiter size diminishes by 50%. In RÖSLER's study (1965), 78% of patients had good and 19% satisfactory results. The incidence of post-therapeutic myxedema was surprisingly low, since only 3% of patients became hypothyroid, an additional 5% presenting with diminished thyroid reserve. Radiation thyroiditis often occurs on the second or third day of treatment and can be quite painful. It responds well to prednisone.

After any form of treatment of goiter, lifelong medication with 200 µg of L-thyroxine/day is indicated. The recurrence rate, which is 15% in untreated cases (JENNY, 1966) is reduced to a minimum by thyroxine (BERGFELT, 1963). Persisting TSH stimulation after surgery is probably responsible for the high incidence of malignant tumors in recurrences of originally benign goiters (EGLOFF, 1961).

2. Endemic Cretinism

a) Definition

The Pan-American Health Organization has adopted the following definition for this syndrome: "The term endemic cretin describes mentally deficient subjects born in an endemic goiter area, who exhibit some of the following characteristics which are not readily explained by other causes: 1. Irreversible neuro-muscular disorders. 2. Irreversible abnormalities in hearing and speech which sometimes lead to deaf-mutism. 3. Impairment of somatic development. 4. Hypothyroidism." This definition is by and large identical to the one DE QUERVAIN and WEGELIN (1936) have given in their classic monograph and which has been used by KÖNIG (1968) in a modified form (Fig. 25).

Thus the only constant clinical feature of cretinism is mental deficiency and in its absence the diagnosis is hard to accept. All other symptoms may be present in very variable degrees which, although all cretins look somehow like siblings, gives each cretin some individuality. In very mild cases the diagnosis can only be suspected and there are no clear-cut criteria or laboratory tests by which borderline

cases can be separated from normal persons. It is still a matter of debate whether endemic iodine deficiency can cause hearing loss without concomitant mental and physical retardation. Although there are no good convincing theoretical arguments to refute this possibility, such patients do not fit the definition of cretins given above.

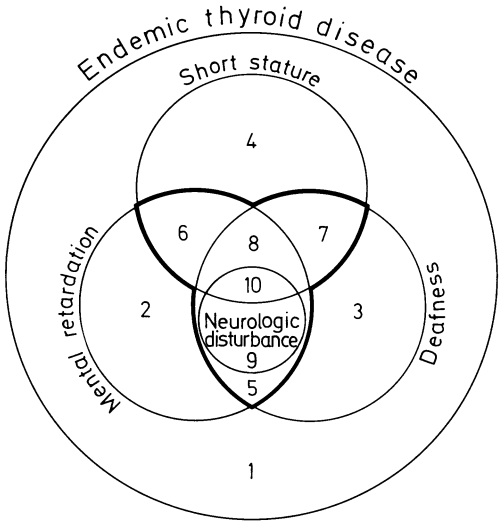


Fig. 25. The clinical features of endemic cretinism. There are at least 10 combinations of the four main signs. The diagnosis of cretinism is usually very difficult to establish in monosymptomatic cases (From KÖNIG, 1968)

Some authors think congenital athyrotic hypothyroidism (p. 166) and endemic cretinism are difficult to separate, but we agree with DE QUERVAIN (1936) that the two diseases are basically different. There is an overlap of many symptoms but differentiation is generally possible on clinical grounds. While the endemic cretin is born as such, with irreversible damage to the hearing organ and to the brain, mental and physical development is only slightly retarded in the congenitally athyrotic child. His height is near-normal in most cases, although bone maturation is delayed. His alertness is reduced, but there are no hearing loss or other signs of neurologic damage.

Thyroid hormone substitution therapy is of little value in improving the central nervous system manifestations of endemic cretinism, although some effect on growth, skeletal maturation and the development of puberty is possible. In contrast, much depends on early substitution therapy in congenitally hypothyroid children, and the brain damage can often be kept to a minimum. When left untreated, congenitally hypothyroid children come to resemble endemic cretins more and more. The differentiation between endemic cretinism and congenital hypothyroidism was first clearly established by DE QUERVAIN and WEGELIN (1936). Their arguments and their signs of differentiation are still valid today. We must acknowledge that in the more recently described area of Idjwi Island, where 90% of cretins are hypothyroid, the endemic cretins can hardly be distinguished from sporadic congenitally hypothyroid patients (DELANGE, 1972). To avoid confusion, it has been suggested that the term "sporadic cretinism" be dropped and replaced by "congenital hypothyroidism".

b) Geographic Distribution and Incidence

Endemic cretinism occurs only in areas where goiter is severely endemic. It is never observed near the sea coast. Some endemic goiter areas, such as the Great Lakes in the U.S.A., have been found to be free of endemic cretinism. Table 15 gives the incidence of endemic cretinism as obtained in surveys over the past 90 years in various areas where goiter is endemic.

Cretinism has been familiar for many centuries in the Alps (Savoy, Piedmont, Steiermark, Salzburg area), in the Pyrenees, the Carpathian mountains, the Caucasus, southern Sweden, Hungaria and Yugoslavia. During the past 70 years endemic cretins have been detected and well studied in many parts of Asia (Himalayas, Sumatra), Africa (Uele, Idjwi Island), and New Guinea (Mulia valley), and in parts of the South American Andes (Gujas, Brazil; Ecuador; Peru) (Table 15).

Table 15. Incidence of cretinism in various endemic areas as established by surveys during the last 35 years. (From KÖNIG, 1968)

Mulia, New Guinea	8.2%	CHOUFOER (1965)
Gornia Josanica, Yugoslavia	6.7%	KICIC (1961)
Blumenstein, Switzerland	3.5%	EUGSTER (1938)
Idjwi, Zaire (Congo)	1.0%	DELANGE (1966)
Canton Aargau, Switzerland	0.6%	EUGSTER (1938)
Uele, Zaire (Congo)	0.1–0.3%	BASTÉNIE (1962)
Piemont, Italy	0.01%	COSTA (1964)
Piemont, Italy	0.15%	Survey of 1883

The incidence of cretinism is very difficult to estimate in a given focus of endemicity, because the symptomatology is so variable. There is a smooth transition from normal persons over mild cretins to severe cretins and it is left very much to the judgment of the individual observer where he wants to draw the dividing line. In Mulia (New Guinea) the incidence was 8.2%. In Idjwi (Republic of

Table 16. Relative incidence of cardinal symptoms of endemic cretinism in various areas. (From KÖNIG, 1968)

	Mental retardation	Deafness	CNS defects (spasticity)	Retardation of bone development	Goiter	Hypothyroidism	Severity of endemic goiter
Uele (Zaire)	Most cases	Occasional	Occasional	Most cases	Occasional	Most cases	Severe
Idjwi (Zaire)	Most cases	Occasional	Occasional	Most cases	Occasional	Most cases	Severe
Mulia (New Guinea)	Most cases	Frequent	Frequent	Rare	Occasional	0	Severe
Himalaya	Most cases	Most cases	?	Occasional	Most cases	0 (?)	Severe
Brazil	Most cases	Occasional	Most cases	Occasional	Frequent	Rare	Moderate
Piemont (Italy)	Most cases	Frequent	?	Occasional	Frequent	Rare	Moderate
Canton Berne (Switzerland)	Most cases	Frequent	Rare	Occasional	Occasional	Occasional	Moderate
Steiermark (Austria)	Most cases	Frequent	Rare	Occasional	Occasional	Occasional	Moderate

Most cases: over 90%; Frequent: over 50%; Occasional: over 10%; Rare: under 10%.

Zaire) it was 0.1%. Both sexes are equally affected, as in endemic goiter, which has an equal incidence in prepubertal boys and girls. The symptomatology shows wide regional differences. In the Himalayas and in parts of Latin America, for example, "nervous" cretinism with spastic diplegia predominates, a form which is rare in Europe (see below, and Table 16).

Endemic cretinism is a vanishing disease in the Alps. In the Canton of Berne, where the nursing homes used to be crowded with cretins, all existing cretins are now aged 55 or more. Only a few cretins have been born during the past 45 years in Steiermark (Austria), and no endemic cretins have been born in the Alps for the past 25 years (KÖNIG, 1968). A decrease in the incidence of endemic cretinism was observed in the late 19th century, long before iodine prophylaxis was introduced. In England the disease had disappeared by 1870 (FAGGE, 1871). In Switzerland endemic cretinism started to decline before iodinated salt was introduced. This is probably due to a change in dietary habits with the importation of iodine-rich food. As discussed by QUERIDO (1972), the studies of WESPI (1945) leave absolutely no doubt that the iodination of salt has been the main factor responsible for the eradication of this disease.

c) Etiology and Pathogenesis

Endemic cretinism only occurs in areas of severe iodine deficiency. The urinary iodine excretion (roughly equal to the iodine intake) is under 15 µg per day in most areas where cretins are born (QUERIDO, 1972). As in the case of endemic goiter, goitrogenic compounds of food and drinking water may also play some part. Mothers of endemic cretins always have a goiter and have lived in an area where it is endemic at least during the pregnancy. In a focus of endemicity, cretinism is often clustered within a few families (EUGSTER, 1938; KÖNIG, 1968). However, hereditary factors certainly play only a minor role. Mothers who have given birth to several cretins have normal children after moving to a village where the condition is not endemic (DIETERLE, 1952); conversely, families who move into an endemic focus have the same incidence of cretinous children as the native population (EUGSTER, 1938). In a series of siblings the younger ones are usually more severely cretinous.

The exact pathogenesis of cretinism remains unknown. The most likely hypothesis is that the iodine deficiency interferes with the early function of the fetal thyroid gland. Between the 80th and 90th days of fetal development, follicular lumina appear in the previously solid thyroid tissue, and thyroglobulin and hormone synthesis get under way. At approximately the same time, TSH and thyroxine become detectable in fetal blood. The concentration of thyroxine in the maternal circulation bears little relation to the concentration in the fetal circulation (GREENBERG, 1970), and it appears that the fetus is dependent on its own hormone production (as discussed on p. 151) and receives little, if any, hormone from the mother. Even if the dietary iodine available were enough to maintain borderline euthyroidism in the mother, the fetus might pass through a phase of perhaps only slight hypothyroidism. At a critical time this could cause irreversible damage to the developing central nervous system.

In later fetal life, lack of thyroid hormone predominantly affects growth and bone maturation. These changes are reversible by thyroid hormone replacement.

This theory is rendered less acceptable by the fact that fetal thyroid hormone lack due to other causes (such as thyroid aplasia) does not produce deafness or irreversible brain damage. Several children in whom hormone replacement was started during the first days

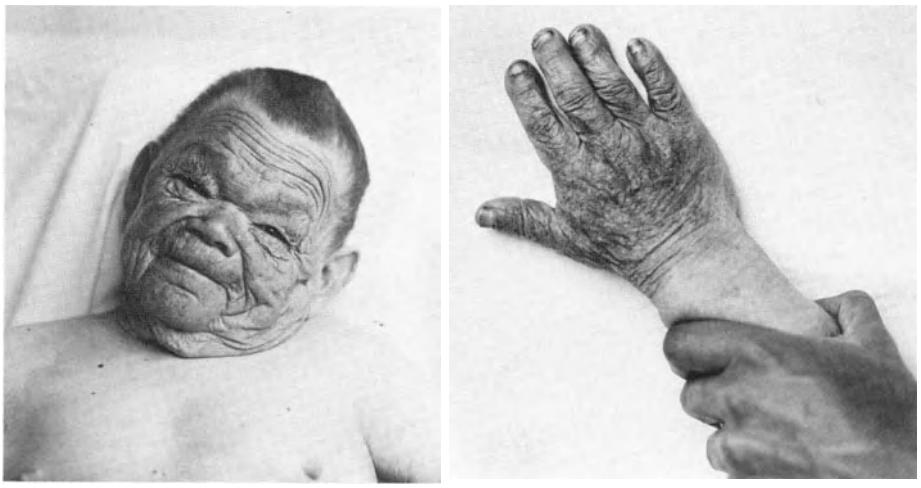


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Fig. 26a and b. 69-year-old man. Typical cretinism of alpine type (Collection Prof. D. LOOSER, Winterthur)

of life have been carefully followed and found to have a normal mental development. Only if left untreated do such children become retarded and finally resemble true endemic cretins, but even then nerve deafness is a very rare finding. Alternative mechanisms have therefore been proposed. Some authors think the damage to the inner ear takes place in very early pregnancy, before there is any discernible fetal thyroid function. They think an iodinated compound different from thyroid hormone is necessary for normal maturation of the central nervous system. Moreover, euthyroid cretins often have much more extensive neurologic damage than severely hypothyroid cretins (DELANGE, 1972).

In summary, the pathogenesis of the neurologic deficits of cretinism is only partly explained by a lack of fetal thyroid hormone. Other mechanisms must also be involved.

d) Clinical Features of Endemic Cretinism

All grades are seen, from nearly normal to severely affected individuals. The severity of cretinism is best graded by the degree of mental retardation. In mild cases the patients are able to talk, read and write, and can perform simple tasks. Mild cretins have married and have had children. Moderate cases are capable of communicating with other people with the aid of gestures and a few words. They can only perform very simple work. Severely affected patients are completely deaf-mute and incapable of any work. They need permanent care in nursing homes.

Cretins often have a very *typical appearance* and they have been distinguished from other types of mentally retarded subjects for centuries. They have a round face, generally with an amiable serene expression (Figs. 26, 27) and they often arouse sympathy in other people. A few cretins are depressed or even aggressive (Fig. 28). The slow growth of the skull base with persistent synchondrosis between the sphenoid and occipital bone produces the depressed nasal bridge with apparent protrusion of the cheek and jaw.

The hairline is low, the scalp hair is coarse and fur-like, beard growth is sparse and the

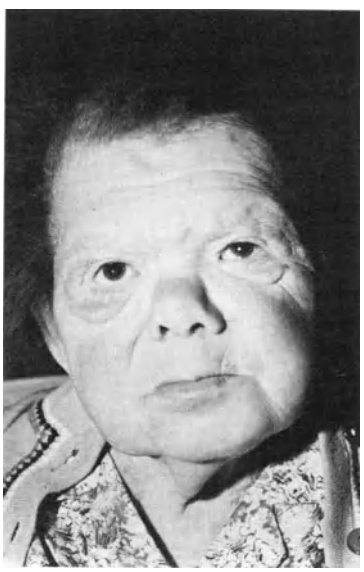


Fig. 27. 68-year-old cretin woman with severe myxedema. The patient had remained infantile in mental development. She was able to read and write and to do needlework. The intelligence age was estimated at 12 years. The patient was affectionate, amiable and grateful. She was the pet of the nursing staff. Height 118 cm, weight 28 kg. Note that she looks much younger than her age and has no gray hair

skin is wrinkled and myxedematous. In typical cases the diagnosis can be made on the spot. In any area, most cretins look like siblings from one big family. Cretins do not look young, nor does age show upon them (Figs. 27, 28). This classic description of the "alpine" type is not applicable to cretins in the Andes, New Guinea, the Himalayas and Africa, where neurologic symptoms often predominate (see below).

Fig. 28. 77-year-old cretin with severe mental retardation. The patient could not talk. The intelligence age was about 3 years. She was suspicious and hostile toward the nursing staff. Height 112 cm, weight 29 kg. Moderate-sized goiter. Slight hypothyroidism

Mental and neurologic damage can assume any degree of severity. Often, but not always, mental and physical disability are parallel. The decreased intelligence, combined with affective and emotional changes and with hypothyroidism, produces a characteristic psychological picture. Cretins are good-humored, sometimes even witty. Their sense of orientation is well developed but they have no notion of numbers or combinations. The sexual drive is diminished in most cases, although occasionally cretinous women have conceived children.

The incidence of deafness varies greatly from one focus of endemicity to the other (Table 16). Its pathogenesis is poorly understood. The pathologic changes in the ear have recently been reviewed by KÖNIG (1972). Deafness is primarily of the nervous type, but middle-ear changes due to faulty bone development are also a contributory factor. Speech can be totally deficient or reduced to a few badly articulated sounds. In mild cases language is quite well developed but the vocabulary remains limited and the voice retains a monotonous intonation.

Mental deficiency is by definition a feature of endemic cretinism. Many observers have the impression that in iodine-deficient areas it may be present with a very high incidence as an isolated sign without the other stigmata of cretinism. In Ecuador, FIERRO-BENITEZ (1972) has shown that children conceived shortly after the introduction of iodine prophylaxis had a higher average IQ than children born during the same period in a neighboring village without prophylaxis, even when obviously mentally deficient subjects were excluded from the study. There was some evidence in this study that iodine supplementation must be started during the first 5 months of fetal life to be effective. However, there is no general agreement as to when exactly the irreversible brain damage takes place. Other investigators have placed the critical time in late fetal life or even the first months after birth.

In some areas such as the Himalayas, New Guinea and Idjwi Island, neurologic symptoms only rarely described in the Alpine cretins predominate. These cretins suffer from spastic pareses of the lower, sometimes also of the upper extremities, with a high degree of motor incoordination resembling that in cerebral palsy.

The significance of *short stature* has probably been overemphasized. Many cretins are near normal in height. In Swiss cretins height of 140 to 150 cm was quite typical. One quarter of European cretins were normal in height and only 7% were shorter than 100 cm. The degree of dwarfism probably parallels the severity of hypothyroidism, and goitrous cretins (see below) with normal thyroid function are often of normal height. Height is said to parallel the goiter size, which probably means that dwarfism parallels the degree of hypothyroidism (see below). As in congenital hypothyroidism, dwarfism is disproportioned, with short extremities. Skull size is small in absolute terms, but is normal relative to height.

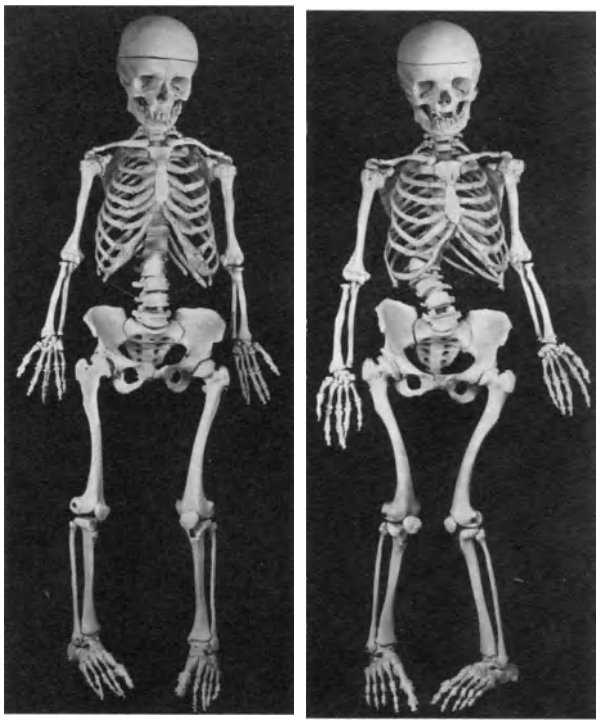
Many signs of endemic cretinism are dependent on the degree of concomitant hypothyroidism. Due to motor incoordination, muscular weakness, and cretinous dysplasia of the hip cretins have a slow waddling gait. Spasticity of the legs may occasionally make cretins incapable of walking.

The skin is often thickened, dry, and scaly as in simple myxedema, sometimes with a

brownish hue. After puberty, myxedematous skin changes often regress and leave a coarse wrinkling which, especially on the forehead, contributes much to the typical appearance of cretins (Fig. 26a).

Epiphyseal growth zones may remain open throughout life. In contrast to the clumsy appearance of the cretin while alive, the skeleton reveals a rather light body build (Fig. 30). Despite this, the hands always look plump due to their shortness (Fig. 26b). The impairment of skeletal maturation produces changes in the hip which are quite similar to those in Perthes' disease (Fig. 12, p. 174, Fig. 31). Degenerative changes also occur in the knee. As in congenital hypothyroidism, transversal calcified "growth zones" are seen in the long bones. The humerus may show varus deformity. The musculature is badly developed, which is one of the reasons for the tendency to abdominal hernias. The abdomen is protruding due to muscular weakness and to constipation with flatulence.

There are cretins with goiter and cretins without goiter (Fig. 29). The latter are usually severely cretinous, tend to be hypothyroid, and are shorter in stature. The thyroid gland may be completely atrophic and be replaced by a few fibrous nodules. In such cases hypothyroidism is very frequent. The causes for the atrophy of the gland are not known. It is probably already present at birth. The proportion of hypothyroid cretins varies in different areas where cretinism is endemic (Table 16), but is generally low, except in Idjwi Island, where 90% of cretins are hypothyroid (DELANGE, 1972). In euthyroid cretins the changes in thyroidal function tests are the same as those observed in endemic goiter (p. 213).



a b

Fig. 30a and b. Skeletons of the two patients in Fig. 29. The patients died at age 75 and 77 years respectively. Note the varus deformity of the humerus and the "cretinous hip". (Institute of Pathology, University of Zurich)

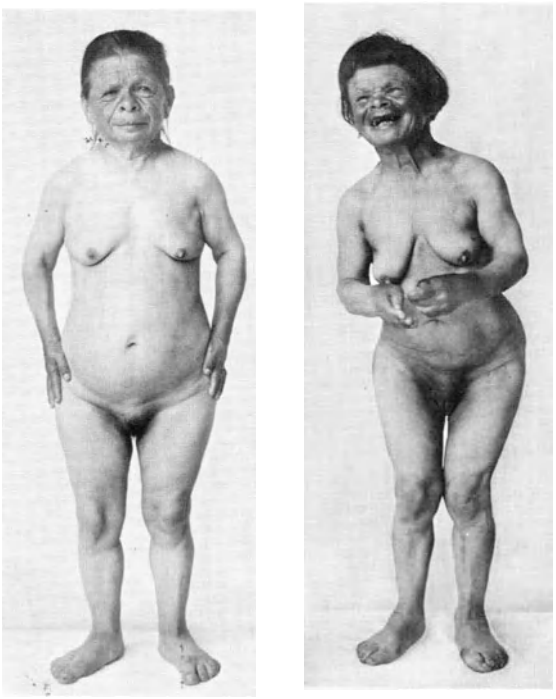


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Fig. 29a and b. Typical cretinism in two sisters, both without goiter. a) 62-year-old woman. 120 cm height. b) 63-year-old woman, 112 cm height. (Collection Prof. O. LOOSER, Winterthur)

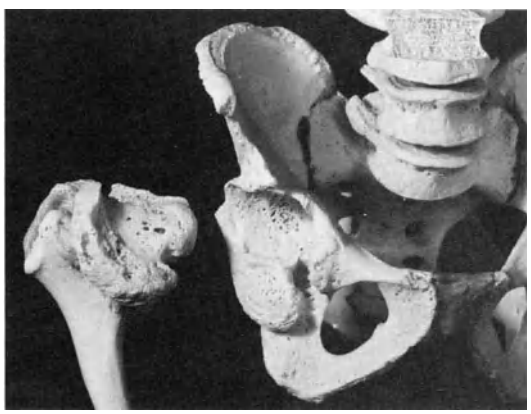


Fig. 31. Hip joint of same patient as Fig. 26, who died at age 80. Severe degenerative hip changes

e) Diagnosis and Differential Diagnosis

In classic adult cases the diagnosis is easy, but in the newborn it can be quite difficult. Children with endemic cretinism have to be differentiated from congenitally hypothyroid children with thyroid aplasia and from those with other forms of mental deficiency, such as Down's syndrome.

f) Course and Prognosis

With good care the life expectancy is virtually normal. Thyroid carcinoma is possibly more frequent in cretins (KÖNIG, 1968), but the cretinous state is not associated with an increased risk of any other potentially lethal diseases.

g) Prevention and Treatment

Prevention is achieved by the same means as that of endemic goiter. Endemic cretinism is eradicated much more rapidly and easily than endemic goiter. In Switzerland no new cretins have been born since the introduction of iodinated salt. Due to a relatively low level of salt iodination, however, goiter remained quite prevalent. There were 30–40 cretinous children in the Canton of Appenzell in 1922. In 1937, 15 years after the introduction of iodinated salt, there were none. The incidence of severe mental retardation among children of school age decreased from 1.4% to 0.4% in the same period. There was a similar fall in the incidence of deaf-mutism in school children (WESPI, 1945).

Treatment of cretins is not very rewarding. Much of the central nervous system damage is irreversible when the cretins are first seen. No data are available on the effects of thyroid hormone therapy started during the first days of life. It is possible that early thyroid hormone substitution could improve the outcome, as in sporadic congenital hypothyroidism (p. 176). In later life thyroid hormone replacement may improve the state of hypothyroid cretins slightly, often at the price of changing a docile patient into an aggressive one.

H. Sporadic Euthyroid Goiter ("Simple" Goiter)

Euthyroid goiter also occurs in areas where the iodine supply is adequate. By definition, euthroid goiter is of course much rarer than in areas where goiter is endemic. It can be multinodular or diffuse and the pathologic findings are exactly identical with those in endemic goiter. The etiology of this form of euthyroid goiter is obscure. In regions where goiter has previously been endemic, and dietary supplementation with a borderline amount of iodine has been introduced, as in Switzerland, it is impossible to decide whether a euthyroid goiter should be classed as endemic (due to persisting borderline iodine deficiency) or sporadic. Euthyroid goiter undoubtedly also occurs when the dietary iodine supply is very generous, as it is in many areas of the U.S.A. In such iodine-replete areas, euthyroid goiter should arouse a much stronger suspicion of malignancy. The pathophysiology of euthyroid sporadic goiter is similar to that of endemic goiter in most respects. The iodine content of the total gland is normal, but the iodine per g gland weight and the degree of iodination of thyroglobulin are low, as in endemic goiter (ERMANS, 1968). It therefore appears that despite adequate dietary iodine supply the gland suffers some form of iodine deficiency; either it cannot accumulate iodide from the blood or transform it into hormone as efficiently as a normal gland, or it loses increased amounts of non-hormonal iodine. The British Medical Research Council (1944) has therefore given the following excellent definition of euthyroid ("simple") goiter: "The immediate cause of simple goiter is failure of the thyroid gland to obtain a supply of iodine sufficient to maintain its normal structure and function. This failure is usually brought about by an absolute environmental deficiency of iodine; it may also be caused by factors which interfere with the availability of dietary iodine or which impose an abnormal demand on the thyroid gland." Biochemical causes of the development of euthyroid goiter despite an adequate iodine supply have of course been looked for. So far none of the gross defects causing familial goiter (see p. 167) has been detected, but subtle defects may well have been overlooked by the techniques available. TUBIANA (1971) has carefully examined the iodine kinetics in 280 cases of euthyroid goiter in the iodine-replete area of Paris. Uptake of both radioiodine and total iodine was higher than in normal individuals. This suggests that the gland is capable of accumulating iodide, but that there is some defect in the intrathyroidal metabolism of iodine.

RAPOPORT (1972) has analyzed the iodine metabolism, the enzymes necessary for hormone formation, and the thyroglobulin structure of a sporadic euthyroid goiter. He found no abnormality typical of the congenital enzyme defects of familial goiter (p. 167).

In a series of 234 cases of so-called nontoxic goiter (a term we try to avoid since it includes hypothyroid and euthyroid goiters) CASSIDY (1970) found that 15% of patients were hypothyroid. In an analogous study from Denmark, AGERBAEK (1973) found a "classic" defect of hormonogenesis in about 10% of patients, while in the remaining 90% no biochemical cause for the goiter was found.

If hormonogenesis is subtly impaired in sporadic euthyroid goiter, the goiter formation must be due to a compensatory rise of TSH levels. Such a rise was actually observed by LEMARCHAND-BÉRAUD (1969).

Clinical features, treatment and prevention of recurrence of sporadic euthyroid goiter are by and large the same as are outlined for endemic goiter (p. 213).

I. Thyroiditis

1. Introduction and Classification

The existence of numerous synonyms for the various forms of thyroiditis has created some confusion in terminology. The American Thyroid Association has recently adopted a standard nomenclature which is presented in Table 17 together with the various synonyms.

Table 17. Nomenclature of thyroiditis according to the American Thyroid Association (WERNER, 1969)

American Thyroid Association	Synonyms
Subacute or acute non-suppurative thyroiditis	Granulomatous thyroiditis Giant cell thyroiditis De Quervain's thyroiditis
Chronic lymphocytic thyroiditis	Hashimoto's thyroiditis Struma lymphomatosa Autoimmune thyroiditis
Chronic invasive fibrous thyroiditis	Riedel's thyroiditis
Acute suppurative thyroiditis	
Chronic nonsuppurative thyroiditis due to specific infection (tuberculosis, syphilis)	

Subacute nonsuppurative thyroiditis and chronic lymphocytic thyroiditis are relatively frequent. All other forms of thyroiditis are extremely rare. The classification given in Table 17 does not include focal thyroiditis, which is quite often seen in large euthyroid (sporadic or endemic) goiters. Focal infiltrates by lymphocytes are often scattered throughout such goiters. Their significance is not known. Similar infiltrates also occur in diffuse toxic goiter (Graves' disease, Fig. 15, p. 182). BASTÉNIE and ERMANS (1972) have prepared a superb authoritative monograph on all aspects of thyroid inflammation.

2. Acute and Subacute Nonsuppurative Thyroiditis

a) Etiology and Incidence

The synonyms for this disease are listed in Table 17. A recent review of the literature has been prepared by GREENE (1971). The incidence of this disease is difficult to establish, and widely varying values have been reported (see GREENE, 1971, for review). All investigators agree that it is much less frequent than Graves' disease and rather less frequent than Hashimoto's thyroiditis. Females predominate in all series, but again the sex ratio varies widely.

The etiology is unknown. Upper respiratory tract infection occasionally precedes the thyroidal inflammation and rises in mumps or coxsackie antibody titers are often recorded. EYLAN (1957) has isolated mumps virus from affected thyroid glands. HINTZE (1964) has observed epidemics of the disease. A virus etiology has therefore often been suggested.

b) Pathology of Subacute Nonsuppurative Thyroiditis

The histological hallmark is focal infiltration with granulomas of histiocytes and giant cells (foreign-body type) associated with a moderate degree of fibrosis (Fig. 32). The granulomas are sometimes difficult to differentiate from tuberculosis. In most cases only a part of the thyroid gland is involved.

c) Clinical Features and Treatment of Subacute Nonsuppurative Thyroiditis

The onset is rather sudden and the patient complains of a painful lump in the neck. Palpation, neck movements and swallowing cause severe pain, often radiating to the jaw or the ears. There is marked general fatigue and fever is common initially. The inflammation starts in a circumscribed area of one lobe, subsides and then resumes in another part of the gland. This wandering of the inflammation is very characteristic and often allows a diagnosis on clinical grounds.

In subacute forms pain may be minimal, and it is difficult to differentiate the enlarged gland from a euthyroid multinodular goiter. Pressure symptoms are quite rare.

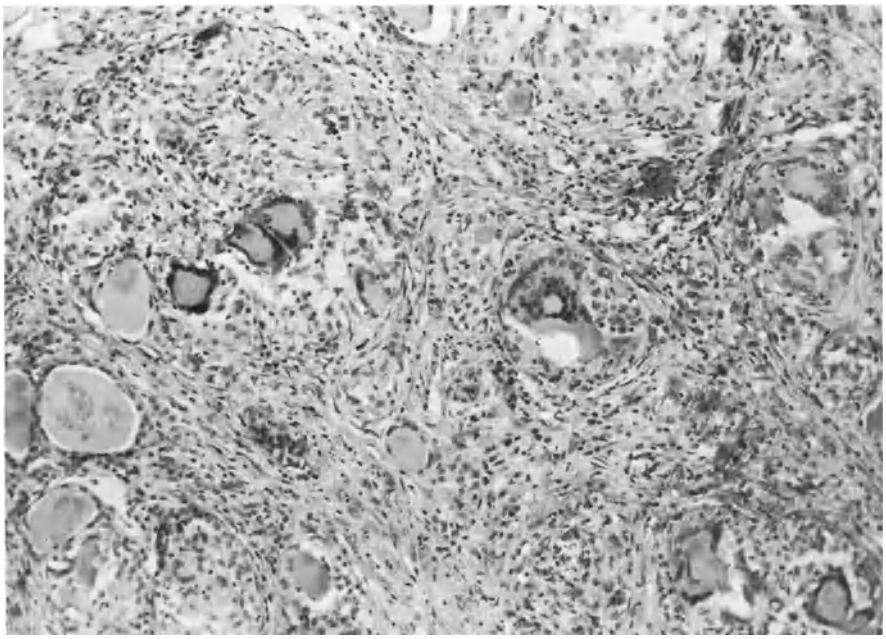


Fig. 32. Histologic appearance of the thyroid gland in nonsuppurative (DE QUERVAIN'S) thyroiditis in a 27-year-old woman. The thyroid tissue is densly infiltrated by granulomas resembling those of tuberculosis. Note the foreign-body type giant cells in contact with remainders of colloid. Hematoxilin-Eosin stain, × 135 (MB 1760/53)

The erythrocyte sedimentation rate is always markedly elevated, sometimes to extreme values. There is moderate leukocytosis with an increase in band forms. Levels of α_2 globulins are elevated. Serum thyroxine is high initially (OGIHARA, 1973, see p. 283) and is sometimes low in later stages. Iodinated proteins leak into the blood and lead to a gross rise in protein-bound iodine. Radioiodine uptake is very low or nil in the beginning, and rebound hyperactivity is seen in the later course. Sometimes the uptake is suppressed only in the affected lobe. Repeated scanning after a few weeks may reveal that the originally suppressed lobe is taking up radioiodine again, while the other lobe is now affected. Thyroid antibodies are not usually present in serum. A rise to low titers is occasionally observed after the disease.

Several diseases have to be considered in the differential diagnosis. Chronic lymphocytic thyroiditis causes less pain or none at all, no fever and no fatigue; moreover high titers of thyroid antibodies in serum are almost always present. Acute suppurative thyroiditis is accompanied by local lymphadenopathy and marked leukocytosis. Pharyngitis, an infected branchial cyst, hemorrhage into a cyst of a nodular goiter, goiter of Graves' disease and thyroid carcinoma have at times been confused with subacute nonsuppurative thyroiditis.

The diagnosis can usually be made on clinical grounds. In doubtful cases it can be established by needle biopsy or the less traumatic fine-needle aspiration (PERSON, 1968). A strong suspicion of cancer is an indication for surgery.

The course of the acute form is usually rapid and self-limiting. Transition to suppurative thyroiditis occurs occasionally in previously goitrous glands. The subacute form lasts several weeks to several months. Patients have been known to suffer from the disease for over a year. Permanent hypothyroidism is extremely rare. Acute nonsuppurative thyroiditis has occasionally been followed by thyrotoxicosis (see CZERNIAK, 1957, for references).

Mild cases are treated with aspirin or phenylbutazone. Moderate or severe cases respond favorably to prednisone. The dose is 40–50 mg/day. Improvement and relief of pain are dramatic. After two weeks the dose is tapered to 15–25 mg, and later to the lowest effective dose. The contraindications and precautions usual in corticosteroid therapy must of course be observed. Treatment can usually be stopped after one or two months, but occasionally it may have to be continued for one year. VAGENAKIS (1970) recommends to stop therapy when the radioiodine uptake of the gland has returned to normal or high values. There is no evidence that thyroid hormone given in addition to prednisone has any beneficial effect. Prednisone treatment probably does not affect the underlying disease process, but it keeps the patient free of symptoms and able

to work while the disease runs its natural self-limiting course.

3. Chronic Lymphocytic Thyroiditis

a) Incidence and Etiology

The clinical impression that the incidence of chronic lymphocytic thyroiditis is rising (McConahey, 1962) has been borne out by a careful epidemiological study from Minnesota (Furszyfer, 1972). The annual incidence rate per 100000 was 6.5 between 1935 and 1944, after which it rose steadily to reach a value of 69 for the period of 1965–1967. The annual incidence of Graves' disease remained constant at 35/100000 throughout this period. Furszyfer considers this increase in incidence as real and not an artefact of improved awareness and better diagnostic facilities. Nève (1972) attributes the reported increased frequency to a widening of criteria used for diagnosis. In the U.S.A, chronic lymphocytic thyroiditis must thus be as frequent as Graves' disease. Although comparable data from continental Europe are unfortunately not available, it is our impression that chronic lymphocytic thyroiditis is much rarer in Switzerland than in the U.S.A. Women predominate in all studies, with ratios varying from 40 : 1 to 10 : 1.

It is fairly well established that chronic lymphocytic thyroiditis is caused by an autoimmune process. Circulating antibodies to components of thyroid tissue are found in most cases (Doniach, 1960). The disease is being associated with other autoimmune disorders, such as pernicious anemia (Schiller, 1967), Graves' disease, rheumatoid arthritis (Becker, 1963; Mulhern, 1966), false-positive tests for syphilis (Shulman, 1964), and others with increasing frequency. The histologic picture is also very suggestive of an autoimmune process. The reasons for the development of an autoimmune process are still poorly understood. Antibodies to thyroid and other tissues are frequently found in relatives of patients with chronic lymphocytic thyroiditis (DeGroot, 1962; Hall, 1962). The basic defect is therefore best described as a genetically determined partial breakdown of immune tolerance.

Chronic lymphocytic thyroiditis has several characteristics in common with Graves' disease. In both instances autoantibodies are present in the serum, although in Graves' disease the titers are usually lower. Some degree of lymphocytic infiltration is also present in the thyroid gland of Graves' disease, and, finally, Graves' disease has been seen in patients with the classic histologic picture of chronic thyroiditis (Fatourechi, 1971). Several pairs of monozygous twins have been reported, in which one twin had Graves' disease and the other had chronic lymphocytic thyroiditis (Jayson, 1967; Doniach, 1967; Chertow, 1973).

The atrophic variant of chronic thyroiditis leads to so-called idiopathic primary hypo-

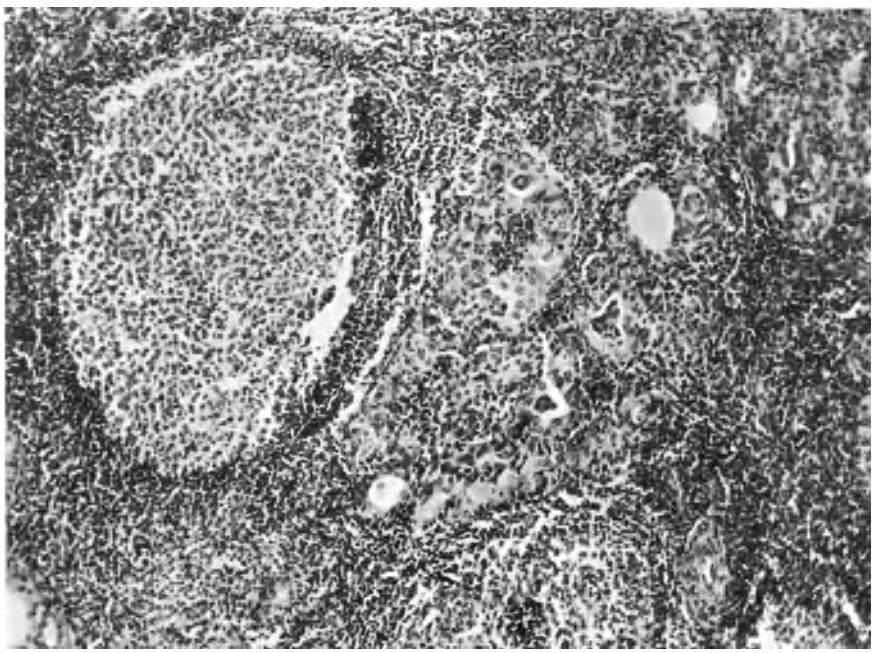
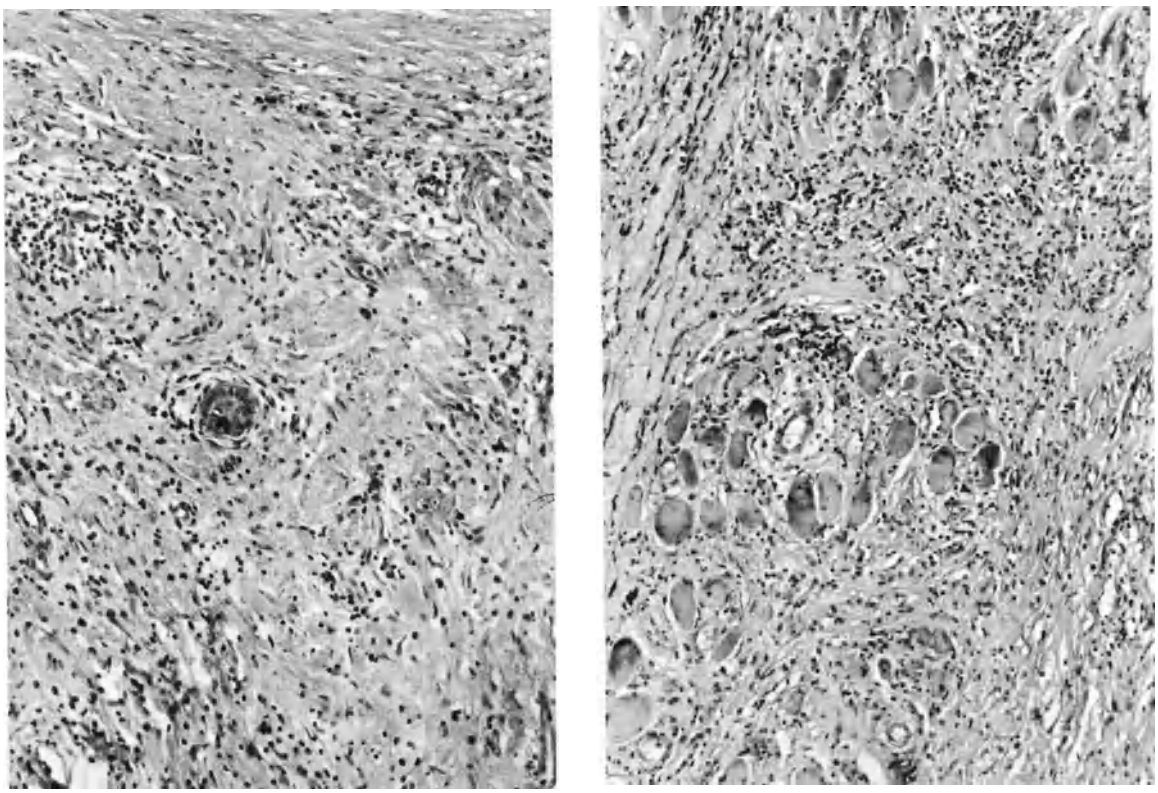


Fig. 33. Histologic appearance of a goiter with chronic lymphocytic (Hashimoto's) thyroiditis. Dense infiltration of the tissue with lymphocytes and lymphatic follicular structures. Woman, age 37. Hematoxilin-Eosin × 135 (MB 11687/54)



a b

Fig. 34a and b. Chronic invasive fibrous (RIEDEL'S) thyroiditis in a 28-year-old woman. a) The thyroid tissue is largely replaced by fibrous tissue and a few lymphocytic infiltrates. b) the fibrous tissue has invaded the surrounding neck structures, in particular the striated musculature. Hematoxilin-Eosin stain, × 150 (MB 3189/60). Note the paucity of lymphocytes compared to Fig. 33 and 35.

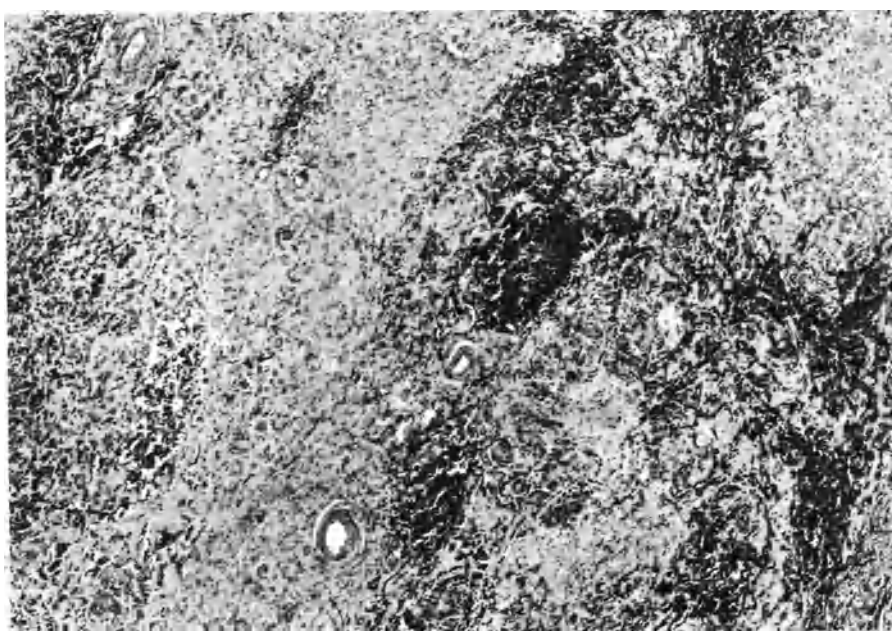


Fig. 35. Atrophic thyroid gland in an 80-year-old woman. The present picture is probably the end result of chronic lymphocytic thyroiditis, atrophic variant. Hematoxilin-Eosin stain, × 65 (SW 1020/62)

thyroidism (p. 153). The whole of the thyroid parenchyma becomes replaced by fibrous tissue (Fig. 35). Basically this variant appears to be due to the same underlying disease process. In both forms there are high titers of thyroid antibodies. The most important difference between the two forms is that in the atrophic variant goiter is absent, while in the classic form it is a necessary criterion for the diagnosis. Some classic cases undoubtedly progress into true atrophic thyroiditis, but how often this happens remains to be established.

In a few cases chronic lymphocytic thyroiditis has progressed into true malignant lymphoma (LINDSAY, 1955; FUJIMOTO, 1967).

b) Pathologic Anatomy of Chronic Lymphocytic Thyroiditis

Focal lymphocytic infiltrates, sometimes with germinal centers, are found in euthyroid (sporadic or endemic) goiters, in toxic diffuse goiters, and sometimes also in thyroid carcinoma. Moreover, moderately high titers of serum antibodies are sometimes present in these conditions. However, these diseases should be strictly differentiated from chronic lymphocytic thyroiditis, in which the lymphocytic infiltration is always diffuse (NÈVE, 1972). The two other criteria which must be fulfilled for the diagnosis are bilateral enlargement of the gland and high titers of serum antibodies.

The infiltrates of chronic thyroiditis consist of lymphocytes and plasma cells. There are numerous germination centers (Fig. 33). The original thyroid architecture is destroyed, with islets of normal parenchyma remaining. Enlarged epithelial cells with an eosinophilic granular cytoplasm (so-called oncocytes, also called Hürthle cells) are often present. Electron microscopy has revealed that these cells are crammed with enlarged mitochondria (NÈVE, 1972). As a rule, multinucleated giant cells are diagnostic for subacute nonsuppurative thyroiditis though they do occasionally also occur in chronic lymphocytic thyroiditis (NÈVE, 1972).

c) Clinical Features and Treatment of Chronic Lymphocytic Thyroiditis

The patients usually presents with a lump in the neck, which has developed over some weeks or months. Goiter is often an incidental finding in a medical examination performed for unrelated reasons. Pain is absent or very mild, but some patients complain of a feeling of pressure. Fever and malaise are absent. Examination reveals a moderately firm goiter, in most cases small. In exceptional cases the goiter becomes very large. Lymphadenopathy is absent. The disease is always diffuse throughout the thyroid, but it may be more prominent on one side. A family history of Graves' disease, goiter or idiopathic hypothyroidism is often obtained. An occasional patient may have typical Graves' disease some years before or after chronic lymphocytic thyroiditis and an unknown proportion of cases develops into severe hypothyroidism due to idiopathic atrophy of the gland.

The laboratory tests reveal normal leukocyte and differential white cell counts. The erythrocyte sedimentation rate is moderately or markedly elevated. The γ globulins of serum are high.

Thyroid function in chronic thyroiditis varies widely. In a group of 51 patients GHARIB, 1972 found that 25 were euthyroid, 22 hypothyroid and 4 hyperthyroid. Association with typical Graves' disease has been reported by FATOURECHI (1972). In cases where the patients become hypothyroid, thyroxine secretion fails first, while near-normal amounts of triiodothyronine maintain a borderline euthyroid state for some time (GHARIB, 1972).

Iodine metabolism shows characteristic changes (BUCHANAN, 1961). The radioiodine uptake is on average within the normal range, but extreme individual variations, from hypothyroid to hyperthyroid values, are typical. The thyroidal iodine pool is always diminished, which leads to an increased fractional iodine turnover and to an elevated PB ^{131}I. The scintiscan may show patchy areas of decreased iodine uptake. The perchlorate discharge test (p. 243) sometimes reveals partial release of trapped radioiodide and suggests some impairment of iodine organification. Due to release of iodoproteins into the blood stream the protein-bound iodine may become elevated despite a normal circulating level of thyroxine. One feature peculiar to the disease is that patients become particularly sensitive to the blocking effects of high doses of iodide, and may become severely hypothyroid when given medication containing iodide, e.g. expectorants for bronchial asthma (BRAVERMAN, 1971; see also p. 154).

The diagnosis of chronic lymphocytic thyroiditis is confirmed by high titers of circulating antibodies to thyroglobulin (so-called tanned red cell test) and complement-fixing antibodies to microsomal antigen. Titers of 1 : 25000 or more in the former test or of 1 : 64 in the latter are virtually diagnostic. Titers of 1 : 2500 or 1 : 16 are suggestive, but may also be found in 50% of patients with Graves' disease. Intermediate or high titers of either one or both antibodies are present in 97% of cases. MORI (1971) used a very sensitive radioimmunologic

technique and found antimicrosomal antibodies in 95% of patients. The diagnosis can be established by biopsy, but the availability of immunological tests has made this superfluous in most cases.

The course of the disease is variable. After a review of the literature, VICKERY (1961) states that about half the cases progress to fibrosis and half remain stationary. In his own 16 cases, which were carefully followed by means of repeated biopsies, 4 showed some increase in fibrosis and 12 remained stationary.

Treatment is indicated in cases with hypothyroidism and when there is a large and troublesome goiter. L-thyroxine in a dose of 0.15–0.3 mg daily will lead to rapid regression of goiter size. A recent long-term follow-up study by PAPAPETROU (1972) has shown that thyroxine treatment does not halt the progressive fibrosis and atrophy of the gland. Administration of thyroxine therefore has to be lifelong. If the patient is euthyroid and is given no treatment he should be notified of the possibility that hypothyroidism may develop insidiously and advised to see his physician at least once a year.

Surgery usually produces permanent hypothyroidism and is indicated only in cases where there is a suspicion of carcinoma.

4. Chronic Invasive Fibrous Thyroiditis (Riedel's Struma)

a) Incidence and Etiology

This is an extremely rare disease which is overdiagnosed by many pathologists, who do not distinguish between this form and atrophic variants of chronic lymphocytic thyroiditis. Originally it was thought that chronic lymphocytic and chronic invasive thyroiditis were the two extremes in a broad spectrum of the same disease, but the two diseases are now separated by clear-cut criteria (see below). Among 42000 patients submitted to thyroid surgery at the Mayo Clinic only 20 cases had chronic invasive thyroiditis (WOOLNER, 1957). Women predominate with a ratio of about 4 : 1.

The etiology is obscure. HARDMEYER'S (1964) original observation that it occurs together with mediastinal or retroperitoneal fibrosis and with TAKAYASU'S arteritis has been confirmed by other authors (RAPHAEL, 1966). HARDMEYER (1964) put forward the hypothesis of a vascular primary lesion.

b) Pathologic Anatomy of Chronic Invasive Thyroiditis

According to WOOLNER (1957) the following criteria describe the pathologic lesion (Fig. 34):

The fibrotic process involves part of the gland, rarely the whole gland. The affected part feels very hard on palpation;

The inflammatory process extends into extrathyroidal surrounding tissues such as neck muscles.

The invasive process completely destroys the affected thyroid tissue. Giant cells are absent.

BASTÉNIE (1972) added two more distinguishing features: the paucity of lymphocytes and the absence of oncocytes.

c) Clinical Features of Chronic Invasive Thyroiditis

Patients affected complain of a rapidly growing goiter which causes severe pressure symptoms and often airway obstruction. There is no fever or malaise. On palpation the thyroid feels very hard ("lignous goiter"). Euthyroidism is the rule. In contrast to chronic lymphocytic thyroiditis, the disease does not respond to thyroxine therapy, and surgery is necessary to alleviate pressure symptoms. After surgery the disease usually subsides or takes a benign self-limiting course. A second operation is rarely required. The preoperative diagnosis in most cases is thyroid carcinoma.

5. Acute Suppurative Thyroiditis

Acute thyroiditis with abcess formation is very rare. Initially it may easily be mistaken for the much more frequent acute nonsuppurative (De Quervain's) thyroiditis. Causative organisms include streptococci, staphylococci, salmonella, E. coli and others.

6. Chronic Nonsuppurative Thyroiditis due to Specific Infection

This form is also quite rare. It may be caused by tuberculosis, syphilis, fungus infection, brucellosis, and occasionally by parasites. It also occurs superimposed on sarcoidosis.

K. Thyroid Neoplasia

1. Benign Tumors

a) Classification

As MEISSNER (1971) has stressed, the numerous subdivisions of follicular adenoma are of dubious value. In most cases the simple classification of the American Thyroid Association (WERNER, 1969, see p. 279) appears entirely satisfactory (Table 18).

Table 18. Classification of benign thyroid tumors.

American Thyroid Association (WERNER, 1969, see p. 279)
Follicular adenoma
Papillary adenoma
Atypical adenoma
Teratoma

b) Follicular Adenoma and Solitary Thyroid Nodules

Follicular adenoma is the most common benign thyroid tumor. It is made up of well differentiated follicles and is indistinguishable from normal thyroid tissue, except that the follicles may be unduly large and the adenoma is surrounded by a capsule. If the adenoma is made up of trabecular structures it is called an embryonal adenoma, if the follicles are very small a fetal adenoma; if the predominant cell type is of the oncocyte variant the terms oxyphilic, or Hürthle-cell adenoma are used. Hürthle cell adenomas are thought by some to be particularly indicative of malignant development (SOLLBERGER, 1957, p. 279), but there is no general agreement on this.

The differentiation of a follicular adenoma from nodules of a multinodular euthyroid goiter is difficult and sometimes impossible. The criteria of WARREN (1953) allow differentiation in many but not all cases (Table 19).

Table 19. Differentiation between nodular hyperplasia and adenoma of the thyroid (WARREN and MEISSNER, 1953)

Nodular hyperplasia	Adenoma
Multiple nodules	Solitary nodule
Nodules badly encapsulated	Well-encapsulated nodule
Variable histologic structure	Uniform histologic structure
Comparable growth rate in adjacent thyroid tissue	Different growth rate than adjacent thyroid tissue
No compression of adjacent thyroid tissue	Compression of adjacent thyroid tissue

The occurence of benign papillary adenomas is doubtful. Tumors with papillary structures are generally malignant with exception of the so-called macropapillary formations in colloid goiters.

Thyroid adenomas often present a functional abnormality since they do not take up radioiodide. The physiologic basis for this has recently been investigated. DE GROOT (1970) found that the "cold" nodules have no iodide-trapping ("iodide-pump") system. DE RUBERTIS (1972) showed that cold nodules were still responsive to TSH when cyclic AMP formation was taken as an index. These studies also suggested a defect of iodide trapping.

A follicular adenoma usually presents clinically as a small soft nodule in the thyroid. It may be detected by an apprehensive patient or as an incidental finding by a doctor in a medical examination performed for other reasons. Lymphadenopathy is absent and euthyroidism is the rule. Some adenomas, however, lead to hyperthyroidism and are called toxic adenomas (p. 204).

A difficult clinical problem is the detection of malignant lesions among the so-called *solitary thyroid nodules* (see Editorial, Lancet, 1964; Editorial, Brit. med. J., 1971). On physical examination, follicular and papillary carcinomas may be indistinguishable from benign nodules, and there are no clinical criteria by which the benign nature of a nodule can be ascertained. It has been said that "cold" nodules, i.e. nodules which do not take up ^{131}I on scintiscans are more likely to be malignant. This is undoubtedly true on statistical grounds, but it does not help in the individual case, since not every cold nodule is malignant and an appreciable proportion of warm or even hot (KENDALL, 1969; GUINET, 1971; FUJIMOTO, 1972) nodules turn out to be cancerous. Scanning with ^{131}Cs may provide some additional information, since practically all nodules cold with ^{131}I and warm with ^{131}Cs are malignant (MURRAY, 1970). Cytological examination of thin needle aspirates may reveal undifferentiated carcinomas, but its value in detecting differentiated carcinomas remains to be established (p. 244).

In practice, therefore, the nature of a solitary nodule is established by a thyroid lobectomy in most cases. This procedure is mandatory in younger people, especially in children, where the likelihood of malignancy within a single nodule was found to be 12.6% (TAYLOR, 1967). At operation a "solitary nodule" often turns out to be part of a multinodular goiter. Surgery is therefore recommended less frequently in areas where euthyroid multinodular goiter is prevalent. In older people the risk of surgery has to be weighed against the likelihood of detecting cancer, although here again, as in children, an appreciable proportion of nodules is malignant.

Any nodule within a multinodular goiter can become malignant (MILLER, 1955). This does not, however, justify the subjection of all patients with a multinodular goiter to

surgery. As WELCH (1966) has aptly calculated, the deaths caused by surgery would outnumber the cases of cancer found.

2. Malignant Thyroid Tumors

a) Classification

Classification of malignant thyroid tumors is still the subject of great controversy. The old continental European classification of WEGELIN (1926), based on detailed pathologic descriptive terms, was unnecessarily complicated. Most of the subclassifications had no clinical relevance. The classification used by British and American pathologists had the great advantage of being simple and more relevant to the clinical behavior of the tumor. However, this classification may have oversimplied the problem, and some tumors found in endemic Alpine areas could not be fitted into any of the Anglo-Saxon entities.

The International Union Against Cancer (UICC) convened an international meeting on thyroid cancer, the proceedings of which were edited by HEDINGER (1969). Much time was devoted to the problem of classification at this conference. A unified tentative system was proposed, but was not officially adopted (UEHLINGER, 1969). More recently, the American Thyroid Association has agreed on another system. It is presented in Table 20 and compared with the classification proposed by EGLOFF and HEDINGER (1964). These authors have carefully reviewed the Swiss histologic material of the last few decades and have greatly simplified the old WEGELIN (1926) classification. As can be seen from Table 20, the two classifications, although not identical, are quite comparable. Controversy exists in fact only over two relatively rare tumors, the metastasizing adenoma and the struma maligna Langhans, which the American authors do not feel merit separate classification. A third tumor, hemangioendothelioma, is never seen in the U.S.A., but it undoubtedly exists in the Alps (see below). Recently WHO in Geneva has proposed a standardized international nomenclature for the histological typing of thyroid tumors (Table 21).

b) Incidence and Etiology

The age-adjusted annual incidence rate per 100000 of thyroid cancer in Finland is 0.8 for men and 2.3 for women. Other countries where goiter is not endemic, such as the U.S.A., have very similar figures (see FRANSSILA, 1971, for review). The relationship of thyroid cancer to endemic goiter is not yet clear. There is no doubt that continuous TSH stimulation in rats or mice (whether produced by TSH injections or by goitrogen feeding) can cause thyroid carcinomas (HERRMANN, 1951). In man there is so far no clear-cut evidence that thiouracil goitrogens cause thyroid cancer (LUNDSGAARD-

Table 20. Classification of malignant thyroid tumors

American Thyroid Association (WERNER, 1969)	EGLOFF and HEDINGER (1964)
Carcinoma	*Epithelial tumors* (cell types may be specified for each tumor: large oxyphilic, cylindric, clear, squamous, spindle, small, giant)
Papillary carcinoma	Papillary carcinoma
Pure papillary	
Mixed papillary and follicular	
Follicular carcinoma	Organoid carcinoma
Pure follicular	Metastasizing adenoma[a]
Clear cell[b]	Struma maligna Langhans[c]
Oxyphil cell (Hürthle cell)[b]	Adenocarcinoma[c]
Medullary carcinoma	Medullary carcinoma
Undifferentiated carcinoma	Anaplastic carcinoma
Small cell	
Giant cell	
Epidermoid carcinoma	
Other malignant tumors	*Nonepithelial tumors*
Lymphoma (specify type)	Malignant lymphoma
Sarcoma (specify type)	Fibrosarcoma, Osteosarcoma
	Hemangioendothelioma[d]
Secondary tumor (specify site of origin)	

[a] The invasive nature of this tumor is not grossly apparent, so-called "low-grade follicular carcinoma".
[b] The justification for this subclassification based on cell types is questionable (see text).
[c] These tumors would be classified as follicular carcinomas in the American and WHO nomenclature.
[d] This tumor is relatively frequent in the Alps. It is only exceptionally seen in the U.S.A.

Table 21. Histological classification of thyroid tumours, WHO Geneva, 1973/74

I. Epithelial tumours
- A. Benign
  1. Follicular adenoma
  2. Others
- B. Malignant
  1. Follicular carcinoma
  2. Papillary carcinoma
  3. Squamous cell carcinoma
  4. Undifferentiated (anaplastic)
     a) Spindle cell type
     b) Giant cell type
     c) Small cell type
  5. Medullary carcinoma

II. Non-epithelial tumours
- A. Benign
- B. Malignant
  1. Fibrosarcoma
  2. Others

III. Miscellaneous tumours
1. Carcinosarcoma
2. Malignant haemangioendothelioma
3. Malignant lymphomas
4. Teratomas

IV. Secondary (metastatic) tumours

V. Unclassified tumours

VI. Tumour-like lesions

HANSEN, 1956). In endemic goiter, a similar continuous TSH stimulation must exist (p. 212). TAYLOR (1971) compared the death rate from thyroid cancer in England to that in Switzerland and concluded that the figure was ten times higher in Switzerland. It is doubtful, however, whether the data from the two countries were entirely comparable. Based on other data FRANSSILA (1971) arrived at an annual mortality rate per 100000 of 0.5 for men and 1.1 for women for thyroid cancer in Finland. On the east coast of the U.S.A., the corresponding figures are 0.3 and 1.6 and in Switzerland 1.5 and 1.6. Thus Switzerland, where goiter was endemic until very recently, has an elevated mortality rate only for men, while the figure for women is comparable to that in areas where the condition is not endemic. Studies from the pathology institutes of Zurich (KIND, 1966) and Berne (WALTHARD, 1963) do not so far substantiate the claim that iodine prophylaxis has greatly reduced the overall incidence of thyroid cancer. They do clearly show, however, that iodine prophylaxis has caused a shift from the less differentiated forms to the more differentiated and less malignant papillary carcinomas, with the overall incidence remaining more or less constant. KIND (1966) has noted a sevenfold increase in the incidence of papillary carcinoma in Zurich between 1940 and 1964. EGLOFF (1961) found a notably high incidence of thyroid carcinomas in patients thyroidectomized years before for an originally benign goiter. This suggested that the TSH stimulation after thyroidectomy contributed to the development of cancer. Few measurements of TSH levels in patients with thyroid cancer have been published. VALENTA (1968) found values that were on average higher than in control persons.

Ionizing radiation is a recognized cause of thyroid cancer. Children appear to be particularly susceptible. This has been conclusively shown in two instances. Thyroid neoplasms (benign and malignant) had an increased incidence in children exposed to radioactive fallout from atomic explosions (SOCOLOW, 1963; CONARD, 1966, 1970) and in children treated with X-ray irradiation for "thymic hyperplasia" (JANOWER, 1971; DE GROOT, 1973, p. 283).

LINDSAY (1965) found several malignant lymphomas of the thyroid in chronic lymphocytic thyroiditis, but CRILE (1962) did not find a similar association

c) Pathologic, Clinical and Prognostic Features

α) Papillary Carcinoma

This is by far the most common malignant tumor in areas where goiter is not endemic, where it makes up roughly 50% of all thyroid malignancies. In endemic areas its relative incidence is less than 10% of all malignant thyroid tumors (WALTHARD, 1963; KIND, 1966). Women are more often affected than men. Papillary carcinoma is the typical thyroid carcinoma of children and young adults. These carcinomas are often very small, 1–2 cm in diameter. Occasionally, multiple cancerous foci are seen within one gland (BLACK, 1960). Initially they are sometimes surrounded by a capsule, but often they show diffuse infiltration of surrounding tissue. Histologically they are made up of a cuboidal or cylindric epithelium arranged in finely ramified papillae. Follicle formation is almost always present. Very typical are the pale, overlapping, so-called ground glass nuclei. The stroma often contains small, calcified spherical bodies, the psammoma bodies or calcospherites. Mixed papillary and follicular tumors generally show the same clinical behavior as pure papillary carcinomas and are classified in the same group (Table 20; LINDSAY, 1960).

Papillary carcinomas metastasize early to the regional cervical lymph nodes (FRANSSILA, 1971). Since they are of low-grade malignancy these metastases were believed for a long time to be "laterally aberrant thyroid tissue" (JOHNSON, 1962). Some authors maintain the opinion

that benign thyroid tissue can occur in cervical lymph nodes (GRICOUROFF, 1962; GÉRARD-MARCHAND, 1964).

Clinical, papillary carcinomas may present as soft, round asymptomatic nodules, so-called *solitary nodules*. The differential diagnosis and management of such nodules have been discussed on p. 228. Older textbooks of medicine or surgery list the following "classic" symptoms and signs of thyroid cancer: rapid growth; firm to hard tumor, which moves badly on swallowing; pain radiating to the ear; recurrent nerve palsy and Horner's syndrome (DE QUERVAIN, 1941). It cannot be overstressed that this is the clinical presentation of advanced undifferentiated cancer as it has frequently been seen in endemic goiter areas. The differentiated tumors which now predominate in areas where goiter is not endemic usually show none of these features. Papillary carcinomas are soft and easily movable, and they cause neither pain nor nerve palsies in the majority of cases. Papillary carcinomas are relatively benign. They remain localized within the neck region for a long time and grow slowly. The survival rate is 75% after 5 years and 50% after 20 years (Fig. 39). Tumors localized within the gland at the first operation have a better prognosis and microscopical tumors have virtually no effect on survival rate (FRANSSILA, 1971; (Fig. 39)). Many papillary carcinomas accumulate iodine and are amenable to radioiodine treatment. Very few papillary carcinomas synthesize thyroid hormones, but in isolated cases they have produced hyperthyroidism (HUNT, 1960; STUDER, 1961; FEDERMAN, 1964).

β) Follicular Carcinoma

About 10 to 20% of thyroid neoplasias are follicular carcinomas. In contrast to papillary carcinomas, these tumors are made up of follicles of various sizes or of combinations of follicles and cords. In contrast to papillary carcinomas, which metastasize to cervical nodes, follicular carcinomas show an early tendency to distant metastases in the lungs and bones (FRANSSILA, 1971). The primary tumor often appears quite benign, which has given rise to the term "*metastasizing adenoma*" in the German terminology. In some series the prognosis of this tumor is equal to that of papillary carcinoma, in others it is slightly worse, although still much better than that of undifferentiated carcinomas (FRANSSILA, 1971; Fig. 39).

Follicular carcinoma cannot be clinically distinguished from papillary carcinoma. Follicular carcinomas usually take up iodine and can be treated with radioiodine.

The term *struma maligna Langhans* applied to tumors of the type originally described by Langhans when goiter was endemic in the Berne area. The criteria included clinical, macroscopic, and histologic features: the tumor always

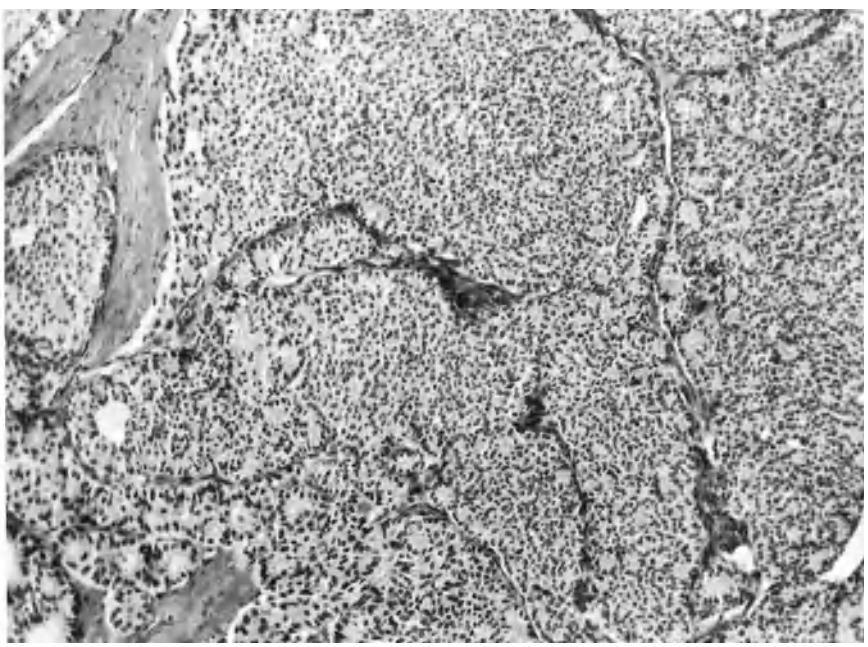


Fig. 36. Follicular carcinoma of the thyroid. The carcinoma is composed in part of trabecular structures. This type would conform to the entity of Struma maligna Langhans of the continental European classification (Table 19). 49-year-old man. Hematoxilin-Eosin, × 140 (MB 1777/55)

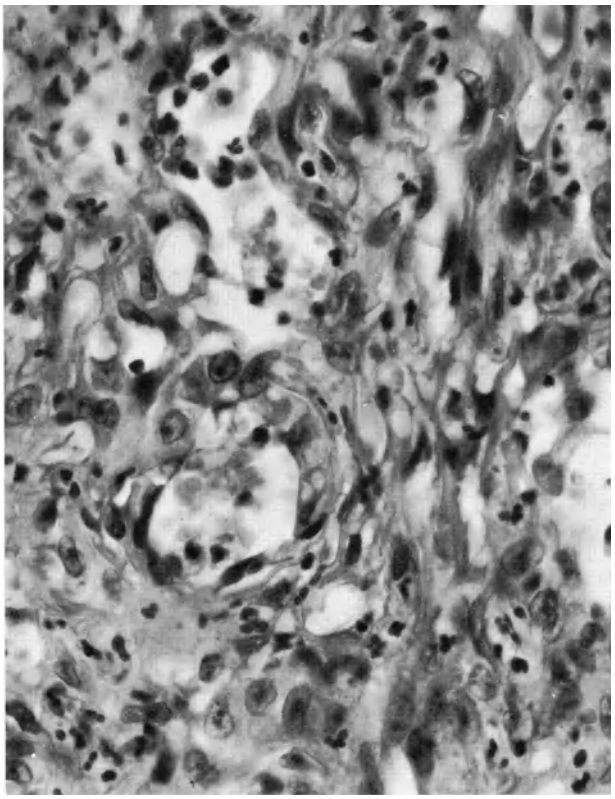


Fig. 37. Hemangioendothelioma in a man aged 77 years. Differentiation of primitive vessels with erythrocytes phagocytized by endothelial cells. Hematoxilin-Eosin, × 300 (MB 1253/53)

arises in a previously goitrous gland; the carcinoma is relatively large (5–15 cm in diameter), well-circumscribed and has a central fibrotic scar. At the periphery it is made up of solid epithelial trabeculae uniform in appearance. In the center it consists of follicles which are much better differentiated than those in simple adenocarcinomas (Fig. 36). Mitoses and vascular invasions are relatively rare. The tumor metastasizes to the lung and bones. After a critical review of the histologic slides of their large Swiss material EGLOFF (1964) maintained the original diagnosis of struma maligna Langhans only in 1/3 of the cases originally diagnosed as such. The remaining 2/3 were reclassified, mostly as poorly differentiated follicular carcinomas. Adherence to EGLOFF'S rigid criteria makes struma maligna Langhans a rare tumor even in the Swiss study, accounting for perhaps 5% of all thyroid malignancies. According to the American and WHO nomenclature the struma maligna Langhans should be classified as moderately differentiated follicular carcinoma.

The subdivisions into *clear-cell follicular carcinoma* and *oxyphil-cell follicular carcinoma* are probably superfluous (MEISSNER, 1971). The last tumor is also called *Hürthle cell carcinoma* and has been thought to be a separate entity, but HORN (1954) has shown that it has the same biologic behavior as ordinary follicular carcinoma.

γ) Medullary Carcinoma

This is a very special thyroid tumor in many respects. It originates from the C cells, which produce thyrocalcitonin (WILLIAMS, 1965, 1966). It is an abundant source of this hormone and has allowed elucidation of its amino acid sequence (NEHER, 1968). Histologically, large amyloid deposits are very characteristic. The cells contain secretory granules (MEYER, 1968; TUBIANA, 1968). Calcitonin levels in blood are elevated. The disease runs in families and affected members can often be detected by elevated blood calcitonin levels long before the tumor is marcoscopically visible (MELVIN, 1971; JACKSON, 1973). Clinically, the behavior of medullary carcinoma is similar to that of other well-differentiated carcinomas. It grows slowly and metastasizes late (Fig. 39).

Medullary carcinoma is often associated with pheochromocytoma (SIPPLE, 1961; WILLIAMS, 1965, 1966; SCHIMKE, 1968). The patients have an

Fig. 38. Patient with medullary carcinoma of the thyroid. The patient had a typical emaciated appearance and fibromas of the tongue, eyelids and lips. (Courtesy of Dr. I. MAC INTYRE, London)

unmistakable emaciated appearance with fibromas of the lips, eyelids and other parts of the body, perioral and palmar pigmentation and severe acne (Fig. 38). At times medullary carcinoma of the thyroid is associated with pheochromocytoma, hyperparathyroidism and Cushing's disease, a syndrome called type 2 multiple endocrine adenomatosis (STEINER, 1968; MARKEY, 1973).

Many aspects of this peculiar tumor have recently been reviewed by HILL (1973).

δ) Anaplastic (Undifferentiated) Carcinoma

These tumors are extremely malignant with rapid growth and spread to distant sites. They are subdivided according to cell type into small or giant-cell carcinomas. In some tumors the cells are spindle-shaped. In Europe such tumors have been classified as sarcomas.

ε) Non-Epithelial Tumors

Sarcomas seemed to be very frequent in the Alpine pathologic material and very rare in the English and American material. HEDINGER (1969) reviewed the slides of 196 cases diagnosed as sarcoma in Zurich and Lausanne. Only 6 cases withstood his rigid criteria, while the others were all reclassified as anaplastic carcinomas. The relative proportion of sacromas among thyroid malignancies in the Swiss material thus declined from 20% to 0.6%, which is comparable to the incidence in other countries.

Hemangioendothelioma (Fig. 37) is virtually never seen in the U.S.A., and its existence has been doubted (CHESKY, 1953; KLINCK, 1969). In the Alps it is not so rare. It is a distinctive thyroid tumor thought by some to be of vascular origin, by others to be a variant of anaplastic carcinoma. Malignant lymphoma of the thyroid is another rare tumor. LINDSAY (1965) has found an association with chronic lymphocytic thyroiditis.

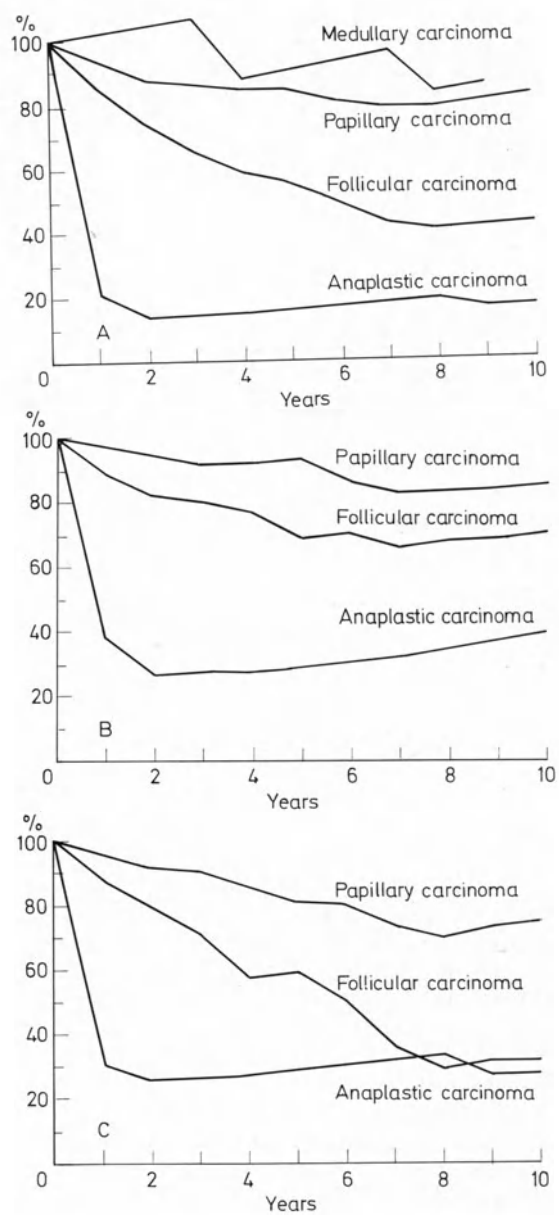


Fig. 39. Relative survival in thyroid cancer according to histologic type. *A* All patients. *B* Patients with intrathyroid tumors. *C* Patients with extrathyroid tumors. (From K. FRANSSILA, 1971)

ζ) Secondary Tumors in the Thyroid

In a careful study SHIMAOKA (1962) found that 8.9% of metastasizing tumors produce secondaries in the thyroid. Metastases are about 10 times more frequent than primary thyroid tumors, but they are rarely diagnosed clinically since they produce no symptoms. There is one exception to this rule. In clear-cell renal carcinoma, metastasis to the thyroid is occasionally the first manifestation of the tumor. It may initially be wrongly diagnosed as a clear-cell so-called postbranchial tumor of the thyroid (HEDINGER, 1967).

d) Treatment of Thyroid Carcinoma

A considerable proportion of thyroid carcinomas retain iodine and are at least partly dependent on TSH stimulation. This permits an unusually wide range of possible methods of treatment from large doses of radioiodine to suppression of TSH secretion by thyroxine administration.

The therapeutic attitude to be adopted in cases of differentiated thyroid cancer is a matter of dispute. Many of these tumors retain responsiveness to TSH, so that it is universally accepted that lifelong suppressive therapy with thyroxine is indicated. Attitudes to additional measures vary between the extremes of hemithyroidectomy followed by a passive "wait and see" and the most vigorous approach consisting of total thyroidectomy with radical neck dissection followed by repeated massive doses of ^{131}I and external radiotherapy. There are good arguments in favor of both approaches and the decision is left to the temperament of the individual physician. Reliable data based on carefully controlled prospective studies are not available. The proponents of the conservative approach argue that differentiated tumors have a slow growth potential and that their approach gives survival rates comparable to other methods (CRILE, 1964, 1966). The patient should therefore be spared the disfigurement of radical neck dissection. The supporters of the other extreme argue that generalization of thyroid cancer is unpredictable and that when it has occured, it is too late for ^{131}I therapy and other measures.

In Zurich we have chosen the following plan for management of thyroid cancer. If a diagnosis of cancer is made, total thyroidectomy is performed, leaving the recurrent nerves and the parathyroid glands intact. Total thyroidectomy is performed in all cases, regardless of whether the disease is bilateral or only unilateral. There are two reasons for this: 1. Many carcinomas are multicentric or have local metastases within the thyroid (BLACK, 1960; ROSE, 1963; WELCH, 1963); 2. Total thyroidectomy is an essential condition for ^{131}I therapy, since any normal thyroid tissue remaining would subtract radioiodine from the malignant tissue. Moreover, malignant tissue only accumulates adequate amounts of ^{131}I if it is properly stimulated by TSH. The hypothyroidism produced by thyroidectomy is a potent stimulus to endogenous TSH secretion.

In cases where the histologic diagnosis of carcinoma is made after the operation a second operation is performed. Local metastases in the adjacent tissue and in the regional lymph nodes are removed, but radical neck dissection with sacrifice of sternocleidomastoid muscles, the accessory (11th) nerve and the internal jugular arteries is not justified. Total thyroidectomy must also be performed in cases where distant metastases are present. POCHIN (1967) has shown that over 50% of differentiated tumors amenable to ^{131}I therapy take up the ^{131}I only after total thyroidectomy has produced hypothyroidism and TSH stimulation.

External radiotherapy may be added when the tumor has infiltrated locally and when regional (but no distant) metastases are present. External radiotherapy is badly tolerated during hypothyroidism, while ^{131}I is only effective in hypothyroid patients. Priority is given to ^{131}I and external therapy is given later. The chances of success of ^{131}I therapy are of course much better with differentiated papillary or follicular carcinomas. Anaplastic carcinomas have been found to take up radioiodine only in exceptional cases, and POCHIN (1967) does not give ^{131}I in such cases. We feel that the decision of whether or not to give ^{131}I should not be made dependent on the histologic type of the neoplasms. It has repeatedly been observed that the tumor may show regional differences in histology and in uptake of radioiodine within one patient. The patient should therefore be given the benefit of the doubt and receive ^{131}I. RAWSON (1965) has observed a sufficient ^{131}I uptake by metastases in 65% of cases pretreated with thyroidectomy and TSH.

According to CRILE (1966) more than half the papillary carcinomas in patients under 40 improve or remain stationary under thyroid hormone therapy, but this does not in our view justify the decision to withhold radiotherapy. In the cases which do progress, the unfavorable course is very often noticed too late to institute an effective program with ^{131}I.

Our practical procedure is the following (Table 22): 1 week after total thyroidectomy

the remaining thyroid tissue is eliminated by 60 mCi of ^{131}I and whole-body autoradiogram and scintiscan are obtained. The patient then receives substitution therapy with 80 to 120 μg of triiodothyronine per day. In selected cases (see p. 234) 4000 rad may then in addition be given over 30 days to the neck region by high energy electron therapy (HORST, 1960).

Table 22. Synopsis of radioiodine treatment of thyroid carcinoma

1. Stop thyroid hormone substitution 2 weeks before planned radioiodine therapy.
2. Give 10 IU of TSH i.m. daily for the last 1 to 3 days before radioiodine.
3. 24 hours after last TSH injection give 150 mCi ^{131}I p.o.
4. Resume thyroid hormone substitution therapy 24 hours after radioiodine.
5. Perform whole-body profile or whole-body scintiscan 48 hours after giving radioiodine.

When surgical thyroidectomy is contraindicated, the thyroid is eliminated by 60 to 80 mCi of ^{131}I given 24 hours after 10 IU of TSH. After 24 hours the patient is started on 80 to 120 μg triiodothyronine per day as above. After 6 weeks the triiodothyronine is discontinued and 2 weeks later ^{131}I is given, again 24 hours after 10 IU of TSH.

Exact calculation of the tumor radiation dose is not possible, since the size of the tumor mass is not known. The dose absorbed depends both on the biologic half-life of ^{131}I in the tissue and on the amount of ^{131}I taken up. Since the latter parameter depends on the iodide clearance of the tissue, which in turn depends on the tissue mass, large or small tumors will derive approximately the same radiation dose from a given serum level of ^{131}I. A uniform standard dose is therefore given which is within permissible limits of whole-body exposure, usually between 100 and 300 mCi. POCHIN (1967) recommends 150 mCi.

Radioiodine is given 2 weeks after discontinuation of triiodothyronine and 24 hours after 10 IU of TSH. Triiodothyronine is resumed 24 hours after the administration of ^{131}I. A whole-body profile or scintiscan is taken 48 to 72 hours after the ^{131}I, and the urinary excretion of ^{131}I is monitored over the first 6 days. The serum radioactivity is measured after 5 days. If it is higher than 0.01% of dose per liter, there is probably still some iodine-trapping tissue.

The procedure is repeated at 3-monthly intervals for as long as the above measurements indicate the presence of tumor tissue. One additional dose can be given when all the active tissue has been eliminated. This is permissible since the whole-body exposure after destruction of all iodine-retaining tissue is very low (30–40 rep). The patient is then placed on life-long therapy with the highest tolerated dose of L-thyroxine, usually 200 to 300 μg per day. At long intervals, test doses of 10 mCi ^{131}I may help detect clinically silent metastases.

Side Effects. General malaise is minimal in most cases. After 2 weeks a short episode of radiation thyroiditis may cause discomfort. Occasionally there is inflammation of the salivary glands or the gastric or bladder mucosa. There may be transient amenorrhea. Bone marrow damage with pancytopenia is reversible, but in 5% it may be necessary to discontinue ^{131}I (POCHIN, 1967). Irreversible bone marrow aplasia is rarely seen, and usually occurs in cases with extensive metastases to the bones. Radiation pneumonitis and fibrosis are seen in cases with pulmonary metastases (RALL, 1957). Leukemia developed in 2% of POCHIN's cases (1967), which this author considers an acceptable risk.

To comply with radiation safety regulations, all persons treated with tumor doses of ^{131}I must be admitted to hospital in units specializing in radiation safety measures.

Results of Treatment. As stated elsewhere the prognosis of thyroid cancer depends mainly on the histologic type. Another important determinant of prognosis is the age of the patient. According to RAWSON (1965) the prognosis is poor in young children and old people. In patients aged 10 to 45 years the prognosis of differentiated cancer is good (see also Fig. 39). Pregnancy has no influence on the course (HILL, 1966).

No controlled studies allowing comparison of the various modes of treatment are available. So far, some encouraging results have been published. One third of the patients with metastases survived for 9 years (BLAHD, 1960), and one half the patients with differentiated tumors survived for 10 years (POCHIN, 1967). HAYNE (1963) recorded that 67% of patients with lymph node metastases and 53% of patients with distal metastases survived 5 years.

In a recent excellent retrospective study VARMA (1970) has compared 133 patients treated between 1933 and 1947 with surgery alone to 310 patients treated between 1947 and 1967 with surgery plus radioiodine. In the combined treatment group the overall mortality for the first 8 years was significantly lower. The effect of the combined treatment was particularly good in patients over 40 years of age.

L. Thyroid Function Tests

In suspected thyroid disorder it is customary to confirm the clinical impression by one or two thyroid function tests. A careful history and physical examination will often suffice to evaluate the thyroid function and will obviate expensive tests, especially in patients who are euthyroid. A number of new routine tests have recently been introduced. Fortunately this has simplified rather than complicated the work-up of thyroid patients. The functional state of the thyroid gland can now be reliably assessed by tests in blood samples in the majority of patients and it is rarely necessary to use the more complicated tests, such as radioiodine tracer studies or the basal metabolic rate. One possible exception is toxic adenoma. It can only be diagnosed with any degree of certainty by tracer studies (p. 204).

Not all tests are equally useful for the differentiation between euthyroid persons and patients with thyroid dysfunction. The "*diagnostic power*" of the various tests and their proper selection are discussed in detail on p. 244 and Table 27.

1. Thyroid Hormones in Serum

It is well to recall at this point that both thyroxine (T_4) and triiodothyronine (T_3) in plasma are reversibly bound to plasma proteins by a dynamic equilibrium (p. 4, p. 142). About 99.97% of T_4 and 99.5% of T_3 are in bound form. Most routine methods measure the total amount of circulating hormone. Since the critical parameter for peripheral hormone effect is the free hormone, it would be more useful to measure the free fraction. This is technically feasible, but is still quite difficult (see below). As outlined on p. 4 (Chap. I) however, the total hormone is quite representative of the free fraction in most cases.

The American Thyroid Association has recently standardized the nomenclature and abbreviations for tests of thyroid hormone in serum (SOLOMON, 1972), and this terminology will be used below.

a) Serum Thyroxine, T_4

In most laboratories this is now measured by the convenient competitive protein displacement method (T_4 [D]) (MURPHY, 1965, 1969). The thyroxine is extracted from a 1 ml sample of serum into an organic solvent which is then evaporated to dryness. The dry extract is taken up in a solution containing small amounts of thyroxine-binding globulin and T_4 labeled with ^{125}I or ^{131}I. After a standard incubation time the labeled T_4 bound to the thyroxine-binding globulin is separated from the free labeled hormone, e.g. by an ion-exchange resin. The thyroxine from the patient sample will *displace* a certain amount of the labeled hormone from the thyroxine-binding globulin. The more hormone contained in the sample, the less labeled T_4 will be bound to the protein. The exact amount of hormone present in the patient serum is read from a standard curve obtained from calibrated samples.

The mean normal value is around 8 µg/100 ml (range 5.5–11.0 µg). The method is very specific and is not influenced by any iodine-containing medications or X-ray contrast media. D-thyroxine, which is sometimes used in the treatment of hypercholesterolemia, interferes with the measurement. The method measures *total thyroxine* (free plus bound). It may therefore give misleadingly high or low values in situations where the binding proteins of serum are altered (p. 4, p. 204).

High values with a euthyroid state are thus found in:

Pregnancy, estrogen treatment, oral contraceptive treatment (elevation lasts for 1 to 3 months after discontinuation of the pill);
Congenital elevation of TBG (p. 143);
Hepatitis (p. 143);
Porphyria (p. 143).

Low values with a euthyroid state occur in:
Hypoproteinemia in nephrotic syndrome;
Congenital deficiency of TBG (p. 143);
Diphenylhydantoin (Dilantin) treatment;
Salicylate treatment (p. 143).

There are several other methods of measuring T_4. A radioimmunoassay (T_4 [RIA]) has recently been developed (CHOPRA, 1971). The principle is the same as in the above assay, but T_4 antibodies are used instead of TBG. This eliminates the extraction step and simplifies the procedure.

Procedures where the amount of thyroxine is estimated by chemical iodine analysis are discussed below (PBI, BEI, T_4 by column).

b) Serum Triiodothyronine, T_3

The concentration of T_3 in serum is about 40 to 80 times lower than that of T_4. This concentration was beyond the limits of chemical analysis for a long time. Improved chromatographic techniques and the use of competitive protein binding, as for thyroxine, have made the exact measurement of T_3 in serum feasible (T_3 [D]). In STERLING's (1969) original technique the hormone is extracted from serum into an organic solvent, separated from thyroxine by two chromatographic steps and finally

incubated with thyroxine-binding globulin solution containing labeled T_3. As in the thyroxine assay, the label is displaced by the T_3 from the patient's serum sample. More recently, this relatively complicated procedure has been replaced by a radioimmunoassay using specific T_3 antibodies (see also p. 143 for references). This has eliminated the cumbersome chromatographic separations and the test may soon become widely available (T_3 [RIA]).

There is some controversy about the true normal value. The original STERLING (1969) procedure had a mean normal of 0.220 µg/100 ml. The more recent radioimmunoassays give average normal values of 0.100–0.150 µg/100 ml.

c) Percent Free Thyroxine, $\%FT_4$, and Absolute Free Thyroxine, FT_4

Consideration of the points mentioned above and discussed in detail in Chap. I (p. 4) makes it apparent that it would be clinically more relevant to know the concentration of the tiny free hormone fraction rather than that of total hormone. This fraction is so small that it is not yet possible to measure it directly by any available method, chemical or immunological.

STERLING (1966) has developed an indirect method. A small amount of labeled T_4 is added to the serum sample, which is then dialyzed against a protein-free buffer solution. Only the free hormone can pass the dialysis membrane. After 24 hours the radioactive T_4 that has passed into the dialysis fluid is measured. It usually amounts to 0.03% of the total radioactivity added (% FT_4). When the total amount of T_4 is measured in the same sample by one of the above methods the absolute content of free T_4 can be obtained by a simple multiplication as follows: $FT_4 = \%\,FT_4 \times \frac{T_4}{100}$.

This method of measuring % free T_4 is laborious and not suitable for routine clinical use.

d) Resin T_3 Uptake, RT_3U

The meaning of this fairly simple test is commonly misunderstood. Because of its name, many doctors think it measures the concentration of T_3, which in fact it does not. The test consists of adding T_3 labeled with ^{125}I or ^{131}I to the patient's serum. After a short incubation, during which the labeled T_3 binds in part to the available hormone binding sites of the binding proteins, the free (unbound) hormone is adsorbed onto an anion-exchange resin and the radioactivity on the resin is measured, (hence the name "Resin T_3 uptake") according to the double equilibrium (CHRISTENSEN, 1960):

$$\text{Protein-}T_3\ ^{125}I \rightleftharpoons \text{free } T_3\ ^{125}I \rightleftharpoons \text{Resin-}T_3\ ^{125}I\,.$$

There are numerous modifications of this test, but in the one most commonly used about 25% to 35% of the added radioactivity goes onto the resin in normal sera. Inspection of the double equilibrium above shows that more radioactivity will go onto the resin when few bindings sites on the protein are available. This is the case in hyperthyroidism, since the excess thyroid hormone occupies binding sites that are normally free. A decrease in free binding sites, and therefore a high resin-T_3 uptake, also occurs when the amount of binding protein in plasma is diminished or when the binding sites are occupied by a drug such as diphenylhydantoin or salicylate. In hypothyroidism, due to a decrease of the serum thyroxine concentration, many binding sites are free. A larger fraction of the T_3 ^{125}I will be bound to the protein and correspondingly less to the resin, i.e. the resin T_3 uptake is low. The same result will also be obtained in cases where the number of available protein-binding sites is increased. This is the case in estrogen or oral contraceptive treatment and during pregnancy. The resin T_3 uptake thus has a *direct* relationship to the serum concentration of thyroxine and an *inverse* relationship to the concentration of hormone-binding protein.

By itself, the T_3-resin uptake is not a very sensitive test for the thyroxine concentration and it should therefore never be performed alone. It should always be combined with the measurement of T_4 in serum. The advantage of the test lies in the fact that it allows the detection of the relatively frequent states with altered hormone-binding protein concentration. If the results of the two tests are compatible, the binding proteins of plasma are normal. If, in contrast, one test indicates hypothyroidism and the other hyperthyroidism, there has been some change in the binding proteins. The various possibilities are explained in detail in Table 23.

e) Free Thyroxine Index, Thyroxine-Resin T_3 Index, $T_4 \times RT_3U$

As discussed above, the combined evaluation of the T_4 concentration and the RT_3U allows a correct diagnosis of thyroid function despite variations in hormone-binding proteins of plasma. The combined tests thus give a better idea of the concentration of the functionally relevant *free* hormone. In many laboratories this fact

is exploited more quantitatively and the T_4* is multiplied by the RT_3U. The thyroxine-resin T_3 index thus obtained is an abstract number which correlates very closely with the *free* hormone concentration (OSORIO, 1962; CLARK, 1965, 1967; RAGAZ, 1967). As outlined above, the direct measurement of the free hormone, though feasible, is too complicated for routine use.

By ingenious modifications the measurement of T_4 by displacement has been combined with the resin T_3 uptake into one simple procedure (ASHKAR, 1972; THORSON, 1972). This yields a value closely correlating with the free thyroxine which is independent from changes in the binding proteins ("normalized serum thyroxine", "T_7", "effective thyroxine ratio").

Table 23. Combined use of T_4 and RT_3U measurements for the diagnosis of thyroid function and of abnormalities in plasma-binding proteins

	T_4	RT_3U
Euthyroidism, normal binding proteins	Normal	Normal
Euthyroidism, elevated thyroxine-binding globulin (pregnancy, estrogens, oral contraceptives).	High	Hypothyroid range
Euthyroidism, low thyroxine-binding globulin (nephrotic syndrome, diphenylhydantoin, salicylates, inherited defect)	Low	Hyperthyroid range
Hyperthyroidism, normal binding proteins	High	Hyperthyroid range
Hypothyroidism, normal binding proteins	Low	Hypothyroid range

f) Thyroxine Iodine (Chromatographic), Thyroxine Iodine by Column, T_4I (C)

In these procedures the thyroxine is separated from other serum components by column chromatography and the iodine in the T_4 fraction is measured chemically by a colorimetric reaction. Since the T_4 molecule contains 65% of iodine, the normal values are 65% of those of the T_4 (D), namely 3.5–7.5 µg/100 ml. Although these methods are better than the PBI (see below), since some iodine contaminants are eliminated, they are still not specific enough and have now been largely abandoned.

g) Protein-Bound Iodine (PBI)

This time-honored test has faithfully served a whole generation of endocrinologists. For a long time it was the best single test for assessing thyroid function (BLACKBURN, 1955). The serum proteins are percipitated with zinc sulfate. Since 99.97% of T_4 is protein-bound, it will coprecipitate with the proteins. The precipitate is incinerated and the iodine in the residue is measured chemically by the ceric ammonium sulfate-arsenious trioxide method (BARKER, 1951). Normal values are 3.5–8 µg/100 ml. Since thyroxine is normally the only iodine-containing compound of serum which precipitates with serum proteins, the test is theoretically an excellent measure of the serum thyroxine concentration, but most radiographic contrast media and some drugs contain iodine, which significantly contaminates the PBI. The factors affecting the PBI have been thoroughly studied (DAVIS, 1966; SÖNKSEN, 1968; FOLDENAUER, 1967). Due to this lack of specificity the test has now become obsolete and has been replaced by the more specific T_4 (D) or T_4 (RIA).

Like the other measurements of serum T_4, the PBI was influenced by variations in hormone-binding protein, e.g. it was high during estrogen treatment, etc.

h) Butanol-Extractable Iodine (BEI)

This method was developed in an attempt to improve the specificity of the PBI. The protein precipitate is extracted several times with acidified n-butanol, in which thyroxine is very soluble (MAN, 1951). The iodine is then measured in the butanol extract. Normal values are 2.1–6.5 µg/100 ml. Now that more specific methods are available, the BEI is of historic interest only.

2. Tests of Radioiodine Turnover

Thyroid hormone is one of the very few iodine-containing substances occurring physiologically in the body. This fact, together with the availability of several radioactive iodine isotopes of convenient physical characteristics, has allowed more detailed assessment of function in the thyroid than is possible for other glands. Radioiodine tracer studies were among the best of thyroid function tests until recently, and a "tracer" was performed in practically every patient with any sort of thyroid disease. Specific measurements of thyroxine and triiodothyronine now allow an equally good or even better assessment of the thyroid state, since these new tests are not subject to contamination by iodine-containing compounds, while the radioiodine tracer tests are heavily influenced by iodine contamination (GRAYSON, 1960). Moreover, the *in-vitro* tests are convenient for the patient, since blood can be taken on a

* Before specific T_4 (D) measurements were available, the PBI was substituted for the T_4.

single visit, while two visits are needed for tracer studies. It is therefore not necessary to perform a tracer study in every case of clear-cut Graves' disease if the doctor has decided on drug therapy or surgery. If the hyperfunction is to be treated with radioactive iodine a preceding tracer study is indicated. Radioiodine tracer studies in conjunction with the T_3 suppression test are very helpful in cases of borderline Graves' disease where the clinical examination and the other tests have given equivocal results. A tracer study is strongly recommended in cases where a toxic nodular goiter (toxic adenoma) is suspected. There is no other reliable way of diagnosing this condition. In euthyroid goiter and in cases of "solitary nodules" (p. 228) scintiscans with ^{131}I may be helpful in detecting cold areas, which raise the suspicion of malignancy.

a) Thyroidal Radioiodine Uptake

A tracer amount of ^{131}I (in the form of the anion iodide) is given to the patient per os. The radioactivity is usually 10 μCi if only the uptake is measured and 50 μCi if a scintiscan is also performed. The uptake into the thyroid is measured by an exactly standardized procedure (IAEA panel, 1962 and 1972) with a collimated detector after 2 hours and after 24 hours (in some laboratories after 48 hours). There are good reasons for the choice of the two intervals; the early (2-hour) uptake is particularly affected in hyperthyroidism, while the late (24- or 48-hour) uptake may be within normal limits. In hypothyroidism, clear separation from euthyroid patients is facilitated by the late uptake. The normal values vary from one area to the other (Table 24). They are inversely related to the average dietary iodine intake of the population. In many parts of the U.S.A. the normal values have steadily declined in the past 15 years due to an everincreasing iodine supply in food (PITTMAN, 1969; CHAPLAN, 1971; SACKS, 1972). A similar phenomenon has occurred in Switzerland (Table 24).

Many iodine-containing foods or drugs can cause misleadingly low radioiodine uptakes. These include potassium iodide, which is present in copious amounts in many cough syrups and asthma tablets, organic iodine compounds such as the antiasthmatic iodopyrine (Felsol powder) and the intestinal antiseptic clioquinol (Enterovioform, Mexaform), the coronary dilatator amiodarone (Cordarone) and practically all radiographic contrast media (except the barium sulfate used for visualization of the intestinal tract), and even swimming pool antiseptics (FREUND, 1966). Some of the organic compounds particularly the water-insoluble contrast media, have half-lives of months to years in the body (see ref. DAVIS, 1966, in section on protein-bound iodine).

Table 24. Normal values for thyroid radioiodine uptake in several laboratories

Place and authors	Year of measurement	Thyroid radioiodine uptake (% of dose ± standard deviation)	
		2 hours	24 hours
Birmingham, Alabama (PITTMAN, 1969)	1959	–	28.6±6.5
	1967	–	15.4±6.5
Berne, Switzerland (STUDER, 1962; STECK, 1972)	1960	29±11	53±13
	1969	15± 6	38±14
Hamburg, Germany (HORST, 1959)	1959	19	44

Antithyroid drugs of the thionamide type greatly depress the radioiodine uptake by inhibiting the incorporation of iodide into thyroglobulin. After prolonged treatment, however, the uptake rises again markedly (STUDER, 1972). Some commonly used drugs have a weak antithyroid effect. This is true of the tuberculostatic agent paraaminosalicylic acid. Sulfonamides and cortisocosteroids in very high dosage may depress the radioiodine uptake, but this effect is rarely of clinical relevance.

Thyroid hormone medication decreases the radioiodine uptake by suppressing TSH release. After thyroxine treatment 3 to 4 weeks should be allowed to elapse before radioiodine uptake is measured. Due to its shorter half-life (1 day) the effect of triiodothyronine wears off more rapidly and an uptake measurement may be done 5 to 7 days after its discontinuation.

Sedatives commonly used in thyrotoxicosis, such as phenobarbital or reserpine, do not change the radioiodine uptake, neither does diazepam (CLARK, 1971) which has been extensively tested in this respect. Radioiodine metabolism in anuria and oliguria, and during peritoneal dialysis has been studied by ODDIE (1970). The results are discussed on p. 152.

b) Labeled Protein-Bound Iodine (PB ^{131}I)

The ^{131}I taken up by the thyroid is incorporated into the very large intrathyroidal stores of organic iodine. From these stores the radioiodine is slowly released back into the bloodstream at a rate of roughly 1% per day. The ^{131}I can be detected in the blood as labeled

T_3 and T_4, most of which is of course bound to the specific binding proteins. It can therefore easily be precipitated by acid and measured as PB ^{131}I. The normal range is 0.05–0.3% of the dose per liter plasma 48 to 72 hours after the tracer dose. In hypothyroidism the PB ^{131}I becomes unmeasurable. In hyperthyroidism it is usually over 0.5%/liter. All iodine-containing drugs which inhibit the radioiodine uptake will of course also invalidate PB ^{131}I measurements. A reduction in the intrathyroidal hormone pool (e.g. after thyroidectomy or radioactive iodine treatment) causes an accelerated passage of the ^{131}I through the thyroid gland. An elevated PB ^{131}I is therefore often found in these states, even when the patient is euthyroid.

c) Scintiscanning of the Thyroid

This method allows localization of the radioiodine within the thyroid gland. It has several uses:

It allows the detection of hot or cold areas within a nodular goiter. Hot nodules are suggestive of toxic adenoma and cold nodules arouse the suspicion of cancer.

The size (or weight) of a goiter can be estimated quite accurately from scintiscans (p. 196). It may also reveal substernal extension of a goiter.

Scintiscan is indispensable in cases of congenital hypothyroidism for the diagnosis of thyroid aplasia or ectopism.

The whole-body scintiscan or the more simple whole-body profile allows detection of metastases of thyroid cancer.

3. Thyroid Uptake of Pertechnate (^{99m}Tc)

Pertechnate anion is actively accumulated by the thyroid gland by the same transport system which traps iodide ("iodide pump"). In contrast to iodide, pertechnate is not incorporated into thyroid organic compounds, and it therefore leaves the gland again by diffusion. The ^{99m}Tc has some physical properties which make it an ideal isotope for short-term diagnostic studies. Due to its short half-life the radiation dose delivered to the body is very low. An i.v. injection of 30–500 μCi of pertechnate (^{99m}Tc) is given, and the radioactivity in the neck is measured by an external detector after 20 min. Correction for the extrathyroidal radioactivity in the other organs in the neck makes it possible to calculate thyroidal uptake of ^{99m}Tc. Normally 0.4–3% of the dose is taken up by the thyroid. In hyperthyroidism the uptake is higher. The test has no value in the diagnosis of hypothyroidism (GOOLDEN, 1971; VAN T'HOFF, 1972).

4. Tests of Peripheral Thyroid Hormone Effect

a) Basal Metabolic Rate (BMR)

The basal metabolic rate is defined as the amount of body heat produced under "basal" conditions, usually expressed as kilocalories per 24 hours. FRIEDRICH VON MÜLLER was the first to suspect that heat production was increased in hyperthyroidism. Shortly afterwards MAGNUS-LEVY verified this hypothesis experimentally. The Americans AUB, DUBOIS, and BENEDICT share the merit of having introduced the measurement of the basal metabolic rate into clinical medicine. For decades it was the only reliable test of thyroid function and it is understandable that doctors are reluctant to abandon it.

The crucial point in the clinical use of the BMR is the validity of the so-called normal values. Unlike any other thyroid function test the normal value of the BMR is proportional to the body surface. Moreover, with a given body surface, it may vary due to varying body composition (ROSSIER, 1951; WEDGWOOD, 1955). The body surface can be estimated according to a formula derived by DUBOIS (1936). More recently, the very accurate standards of FLEISCH (1954) have come to be widely used.

The exact definition of what is meant by *basal* conditions is a matter of debate. Some laboratories insist that the BMR be measured before the patient gets up, which necessarily means that the pátient has to stay in hospital overnight. In other centers the BMR is routinely done in outpatients arriving in the laboratory in the morning before breakfast.

Calculation of Normal Values. Age, sex, height, and weight of the patient are recorded and the normal value is computed with the help of the tables of DUBOIS (1936), FLEISCH (1954), or, in the case of children, of TALBOT (1938). The measured patient value is usually expressed as a percentage deviation from the normal values. The normal range extends from −15% to +15%. In our own laboratory in Zurich the mean BMR for 100 healthy men was +0.9% (standard deviation ±5.5%) and for 100 healthy women +0.004% (standard deviation 6.0%) when the standards of FLEISCH (1954) were used.

Open System. This system requires a more complex laboratory technology. It is more accurate than the closed system, but has not been widely used as a routine method. The subject inspires room air and expires into a bag. The volume, CO_2 and O_2 content of the bag are measured and the calorie production is calculated.

Closed System. The patient is connected to a closed spirometer system filled with pure oxygen. The expired gas is passed through sodium carbonate, which absorbs the CO_2, and the gas is then returned to the spirometer tank. The difference between the initial and the final spirometer volume gives the O_2 consumption. The following 7 rules have to be carefully observed:

1. The whole system, including the patient, must be airtight (mouthpiece, nasal clip, tympanic membrane);
2. The valves must be so arranged as to exclude rebreathing of expired gas;
3. The valves and tubes should present no measurable resistance to gas flow;
4. Constant pressure in the mouthpiece must exclude a slow change of the midrespiratory position;
5. Temperature and water saturation of the O_2 in the spirometer must be constant;
6. The O_2 content of the inspired air should not fall below that of normal room air;
7. The patient must be fasting and relaxed and should breath quietly and regularly.

Falsely high values are obtained if rules 1, 2, 3, 6 and 7 are not observed. In most cases they are due to a leak.

Measuring Procedure:

1. The patient must have fasted for 12 hours before the test;
2. Weight and height are measured. Outpatients are left on a bed in a quiet room for 1 hour;
3. The spirometer is filled with O_2. After temperature and water vapor equilibration of the gas the spirometer is connected to the patient;
4. The respiratory movements are immediately recorded and measurements are started after 1–3 min·
5. The whole measurement lasts 5–15 (average 10) min;
6. The diminution of volume in the spirometer is read and the time period is checked;
7. After reduction of the measured O_2 volume to standard conditions (0°, 760 mm Hg, dryness) the calorie production is calculated. With an empiric respiratory ratio of 0.82, 1 liter of oxygen produces 4825 calories. The value is then expressed as percentage deviation from the expected normal value.

The many simple thyroid tests now available have rendered the BMR almost obsolete, although it is a fairly good thyroid function test. The BMR will undoubtedly remain in use as a research tool, but as a clinical routine it will probably be slowly phased out.

b) Ankle Reflex Time

A slowing of the ankle jerk in hypothyroidism can be observed clinically. The speed of the ankle jerk can be accurately measured by suitable recording instruments, and used as a thyroid test. In the most commonly used instrument the foot of the kneeling patient interrupts part of a light beam which is directed onto a photocell. When the foot moves, the amount of light hitting the cell changes and the process can be easily recorded (Fig. 40). The time from the beginning of the hammer tap to half relaxation is measured. Normal values are between 270 and 330 msec. In hyperthyroidism the values are often lower, but the discrimination is not very good. The test is more reliable for detecting hypothyroidism (RIVES, 1965), where the values are in general over 350 msec. IMBACH (1969) has published normal values for children. The reflex time may be shortened in anxious, tense patients (TSCHUDI, 1966) and during hypoglycemia (ZACHMAN, 1967). It may be prolonged in hyperkalemia (WEISS, 1966), diabetes, late pregnancy anorexia nervosa (FOWLER, 1972) and dermatomyositis.

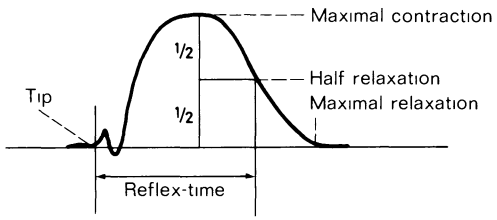


Fig. 40. Ankle reflex registered according to TSCHUDY (1966)

c) Plasma Cholesterol

Plasma cholesterol is undoubtedly elevated in hypothyroidism, probably more so in the primary than in the secondary form (ESCAMILLA, 1953). It has been faithfully passed on as a thyroid test from one textbook to the next. It must be now recognized that there are so many extrathyroidal factors which influence the plasma cholesterol that it should be abandoned once and for all as a thyroid test.

5. Tests of TSH Secretion

a) TSH Radioimmunoassay

LEMARCHAND (1965) and ODELL (1965, 1967) were the first to develop radioimmunoassays for TSH. The technique has meanwhile been greatly improved by work in many laboratories and the clinical usefulness of the TSH radio-

immunoassay is now well established (see HERSHMAN, 1971; MAYBERRY, 1971; Editorial, Brit. med. J., 1971). The normal values vary from laboratory to laboratory as each group uses its own antibody and sometimes its own standard. The most sensitive assays now detect values over 0.5 μU/ml and TSH is measurable in most, though still not all, normal persons (see p. 149 for a more detailed discussion). Values of over 10 μU/ml are clearly above the normal range in most current assays.

The main applications of the radioimmunoassay are: early detection of cases of mild or borderline hypothyroidism ("diminished thyroid reserve", see p. 161), since TSH is clearly elevated when the serum thyroxine is still within the normal range; convenient separation of primary and secondary hypothyroidism; TSH levels are elevated in the primary and depressed in the secondary form.

For the moment, only specialized research laboratories can perform the TSH radioimmunoassay, but the method may soon become widely available.

b) TRH Stimulation Test

The hypothalamic hormone TRH stimulates the secretion of TSH (p. 148). Soon after the determination of its tripeptide structure it was chemically synthesized on a large scale and its diagnostic application has been documented in a flood of publications, only a few of which will be mentioned.

Intravenous injection of TRH causes a rapid rise of the plasma TSH, which reaches a peak between 30 and 40 min and then returns to baseline within 3 hours (ANDERSON, 1971). The response is dose-dependent, but doses upward of 200–400 μg cause a maximal effect (ANDERSON, 1971). A standard dose of 200 or 400 μg is therefore now recommended. TSH rises by an average of 8 μU in males and 12 μU in females (ORMSTON, 1971 a). In hyperthyroidism TRH causes no rise of TSH, while in hypothydroidism there is an exaggerated response (HERSHMAN, 1970; HAIGER, 1971). The test has two main uses: 1. evaluation of pituitary reserve in cases of borderline hypopituitarism. 2. differentiation of pituitary from hypthalamic lesions, particularly in cases of hypopituitary dwarfism (cf. p. 160, COSTOM, 1971).

TRH can also be given orally. It then causes a slower and more prolonged rise in TSH than when given by the intravenous route, but the dose of TRH must be much higher, 1–20 mg (ORMSTON, 1971 b). The prolonged TSH response after oral TRH causes a measurable rise in the serum thyroxine (STAUB, 1971).

c) TSH Stimulation Test

Since the TSH radioimmunoassay is not yet available to all doctors, the time-honored TSH stimulation test will still be used for some time to come. The test is used mainly to distinguish primary from secondary hypothyroidism. The principle is that in primary hypothyroidism the thyroid gland is already maximally stimulated by endogenous TSH and injection of TSH will cause no further stimulation. In secondary hypothyroidism injected TSH causes a marked rise in thyroid activity.

The radioiodine uptake is usually measured as an index of thyroid function. A basal (pre-TSH) 2-hour uptake is measured. Subsequently, 10 IU of bovine TSH (Thytropar, Armour, or 300 U Ambinon, Organon) are injected and a second 2-hour tracer uptake is measured after one day. In primary hypothyroidism there is no rise in the radioiodine uptake. In secondary hypothyroidism the uptake rises by at least 10% and in normal persons by 50%. Repeated TSH injections over 3 days may separate primary from secondary hypothyroidism more clearly (TAUNTON, 1965). The serum thyroxine level usually also rises by at least 3 μg/100 ml in normal persons.

d) TSH Reserve Test with Carbimazole

This test was developed for the detection of cases of diminished thyrotropin reserve (STUDER, 1962, 1964). A basal radioiodine uptake is measured immediately before a one-week course of carbimazole (15 mg t.i.d.) is started. A new tracer dose is given 36 hours after the last carbimazole tablet and the thyroidal uptake is measured again. Due to the TSH secretion provoked by the carbimazole, the second uptake is usually 10–15% higher than the first. This test has not worked well in other laboratories (SCHNEEBERG, 1966; POWELL, 1966), probably due to a higher iodine intake in American subjects. JENSEN (1969) and MORNEX (1970) have much improved the sensitivity of the test by certain modifications. With the advent of the TSH radioimmunoassay and the availability of TRH the test has lost some of its usefulness.

e) Triiodothyronine Suppression Test

In normal persons exogenous triiodothyronine causes a decrease of thyroid activity by suppressing the secretion of TSH. In cases of TSH-independent thyroid function (toxic adenoma, Graves' disease), triiodothyronine does not affect thyroid function (WERNER, 1955).

The basal radioiodine uptake is measured and triiodothyronine (25 µg t.i.d.) is given for one week. In patients with heart disease the dose may be reduced to 40 µg/day for 10–14 days. A second radioiodine tracer is then performed. In normal persons the uptake values decrease by at least 30% (the original uptake being taken as 100%). In thyrotoxicosis there is no decrease of the radioiodine uptake even in borderline cases.

This test is still very valuable in the following circumstances:

1. In the differentiation between an elevated radioiodine uptake caused by iodine deficiency and that caused by Graves' disease;
2. For the establishment of the endocrine nature of exophthalmos in cases of euthyroid ophthalmopathy (p. 202);
3. For the diagnosis of "compensated" toxic adenoma (p. 205). A good review is available on the test (BURKE, 1967).

6. Tests for Inborn Errors of Iodine Metabolism

a) Perchlorate Discharge Test

This test serves to establish the diagnosis of a defect in organic iodination (p. 168). As outlined on p. 140, the iodide transported into the thyroid cell is normally immediately incorporated into organic molecules within the colloid. If this organification fails, iodide accumulates within the gland. If further accumulation of iodide is blocked by perchlorate, the iodide concentration in the gland decreases rapidly because the iodide can leave the gland by diffusion.

A radioiodine uptake is performed and immediately after the 2-hour measurement 0.4–1.0 g of potassium perchlorate is given p.o. The thyroidal radioactivity is measured again 2 and 4 hours later. In normal persons the thyroidal radioactivity remains constant after perchlorate. In defects of organification the thyroidal radioiodide falls by at least 50% (the first value being taken as 100%). Decreases of 10% may indicate partial defects. The test gives a positive (pathologic) result in cases of the familial organification defect (p. 168), during antithyroid drug treatment, in some cases of chronic lymphocytic thyroiditis, and some cases of endemic goiter. In cases of familial goiter the defect should be comfirmed by direct enzyme measurements in the thyroid tissue in all cases where a thyroidectomy is performed (p. 168). (References p. 283).

b) Test for Deiodinase Deficiency

In the familial syndrome, deiodinase is not only deficient in the thyroid, but also in many other tissues, such as liver and kidney. The defect can conveniently be detected in the following way: An i.v. injection with 30 to 50 µCi of ^{131}I-Diiodotyrosine is given and the urine is collected for the next 6 hours. The radioactivity in the urine is analyzed by paper chromatography. Normally the diiodotyrosine is rapidly deiodinated and less than 20% of the urinary ^{131}I is in diiodotyrosine, the remainder being excreted mainly as iodide (ALBERT, 1951; STANBURY, 1956).

7. Miscellaneous Tests

a) Serum Antibodies to Thyroid Tissue Components

These tests are particularly useful in the diagnosis of chronic lymphocytic thyroiditis and of primary idiopathic myxedema. The various antibodies, the corresponding antigens and the tests for their demonstration are listed in Table 25.

Table 25. Thyroid antibodies and methods for their detection. (Modified from KLEIN, in OBERDISSE and KLEIN: Die Krankheiten der Schilddrüse. Stuttgart: Thieme 1967)

1. Colloid antigen 1 (Thyroglobulin)
Agglutination
a) Passive hemagglutination of thyroglobulin-coated tanned erythrocytes.
b) Agglutination of thyroglobulin-coated latex particles or bentonite particles.
Immunofluorescence
Unfixed thyroid tissue sections exposed to fluorescence-labeled serum.
2. Colloid antigen 2 (not identified)
Immunofluorescence (as above)
3. Microsomal antigen
Complement fixation
Immunofluorescence (as above)

Three main antigens are distinguished: thyroglobulin, colloid component other than thyroglobulin and microsomal antigen. In routine practice it is quite sufficient to test for two antibodies (usually antithyroglobulin and antimicrosomal), since a positive response in either one or both tests is obtained in 97% of patients with chronic lymphocytic thyroiditis.

Thyroglobulin antibodies are detected by the sensitive tanned red cell hemagglutination technique. Red cells coated with thyroglobulin are mixed with serial dilutions of the patient's serum and the suspension is examined for hemagglutination. Titers of 1 : 25000 or more are diagnostic for chronic lymphocytic thyroiditis.

The antimicrosomal antibody is detected either by a complement fixation reaction or by immunofluorescence. Titers of 1 : 64 in the complement fixation test are diagnostic for chronic lymphocytic thyroiditis.

Lower antibody titers are found in a number of thyroid disorders, notably in Graves' disease.

b) Measurement of LATS

The significance of LATS and the bioassay method have been amply discussed on p. 179.

The measurement of LATS may be of practical interest in cases of exophthalmos of unknown etiology. As outlined on p. 202, endocrine exophthalmos may occur in euthyroid patients. The presence of LATS in the serum of such patients is strong evidence that they have so-called euthyroid Graves' disease.

c) Thyroid Biopsy, Fine-Needle Aspirates

HAMLIN (1956) and HAWK (1966) have published their experiences with thyroid biopsies performed with the Vim-Silverman needle. A small skin incision is made and the needle is introduced tangentially along the trachea to a depth of 2 cm. The cannula is then advanced and the needle rotated through 180°. A cylinder measuring 1 × 20 mm is obtained. Pressure is applied over the site of biopsy for 5 min. Usually the biopsy causes little pain.

For a long time the main indication for thyroid biopsy was the suspicion of chronic lymphocytic thyroiditis. Most cases can now be diagnosed by the presence of thyroid antibodies in serum.

In suspected cases of thyroid cancer thyroid biopsy is not recommended because of the potential risk of implanting metastases along the needle track. The value of cytological examination of fine-needle aspirates is currently under investigation (GALVAN, 1973).

8. Clinical Indices. Diagnostic Power and Proper Selection of Laboratory Tests

In the great majority of patients hyper- and hypothyroidism can be correctly diagnosed by a careful history and a physical examination. There are two important exceptions to this statement. Both in cases of toxic adenoma and in cases of hyperthyroidism of patients over 60 years of age the clinical diagnosis can be extremely difficult, since often only a few symptoms and signs are present. A wider use of laboratory tests is therefore justified if one of these two conditions is suspected.

Not all clinical features are equally important in the diagnosis of thyroid dysfunction. Some have little, others excellent discriminative function. Several groups have attempted to establish the diagnostic power of each clinical symptom or sign by statistical methods. They have set up "*clinical indices*" in which the presence or absence of each clinical feature is assigned a certain number of plus or minus points. The points for each patient are added up and the sum is compared to a normal previously established range (CROOKS, 1959; BILLEWICZ, 1969; GURNEY, 1970; HARVEY, 1971). Such diagnostic indices are very helpful, especially to the doctor with little experience of thyroid disease. They show which of the innumerable textbook features of thyroid dysfunction are really of clinical importance. The indices have shown, for example, that such commonly taught signs and symptoms as weight gain and constipation

Table 26. Reliability of the most current thyroid function tests for discriminating hypo- and hyperthyroid patients from euthyroid persons. The final impression based on clinical findings and several laboratory tests was considered the correct diagnosis

	Percentage of patients correctly diagnosed			
	Hypothyroidism		Hyperthyroidism	
	FRAGU (1972)[a]	RIVES (1965)[b]	FRAGU (1972)[a]	RIVES (1965)[b]
Thyroidal ^{131}I uptake				
2.5 hours	82.6	–	94.0	–
24 hours	83.6	83	78.4	90
PBI	75.7	92	89.5	87
RT_3U	–	67	–	72
Ankle jerk	86.3	75	84.5	30
Cholesterol	66.0	–	69.0	–
BMR	–	65	–	87
Clinical impression	82.6	–	94.2	–

[a] Normal range adjusted so that in each test an equal percentage of euthyroid people are in the hypothyroid range (false-positives) as there are hypo- or hyperthyroid patients in the normal range (false-negatives).
[b] Normal range adjusted to include 86.5% (±1.5 SD) of euthyroid persons, i.e. each test gives 13.5% false-positives.

in the case of hypothyroidism or hair loss and diarrhea in the case of hyperthyroidism have practically no diagnostic power. Some other conclusions learned from these indices are discussed in the sections on hypothyroidism (p. 159) and thyrotoxicosis (p. 188).

Thyroid laboratory tests must be performed in every case where the clinical examination has given equivocal results. As in the case of clinical features, not all laboratory tests have the same discriminative powers. Several thyroid clinics have determined the value of individual tests by comparing the correlation between the results and the final diagnosis (LUDDECKE, 1958; CHEW, 1962; RIVES, 1965; GORDON, 1970; HARVEY, 1971; FRAGU, 1972). The results of two such studies are shown in Table 26. Of course the value of each test (defined by the percentage of correct diagnoses) depends very much on how the normal range is defined. If narrow limits are given, the test will give few false-negative and many false-positive results, and vice versa. Different definitions of the normal range account at least in part for the discrepancies in the various studies (Table 26).

The *protein-bound iodine* has proved to be one of the best single tests of thyroid function in most studies. No studies comparing the diagnostic value of the newer thyroid hormone measurements (T_4) with that of other tests have yet appeared. But since they are not subject to contamination by compounds containing iodine, an important source of error inherent in the PBI test has been eliminated and the new tests can be expected to obtain a better rating than the PBI, particularly if the T_4 measurement is combined with an RT_3U for the exclusion of disorders of hormone binding to plasma proteins.

The *thyroidal radioiodine uptake* has obtained the same rating as the PBI in most studies. The *BMR* has had an excellent rating

Table 27. Use of thyroid tests in the most common thyroid disorders

	Diagnosis made with reasonable certainty by	Usual additional or confirmatory tests	Tests performed in doubtful cases where diagnosis cannot be established by simpler procedures
Graves' disease	History and physical examination	T_4, RT_3U, possibly ^{131}I uptake and scan.	T_3 suppression test
Euthyroid Graves' disease with ophthalmopathy	History and physical examination	T_4, RT_3U, T_3 suppression test	
Toxic adenoma	Clinical diagnosis difficult. ^{131}I uptake and scan, PB ^{131}I	Repeat scan after T_3 suppression or TSH stimulation	
Triiodothyronine thyrotoxicosis	History and physical examination, T_4, RT_3U	T_3, if not available: BMR; ^{131}I uptake, scan	T_3 suppression test
Primary hypothyroidism	History and physical examination	T_4, RT_3U, thyroid antibodies serum TSH or TSH stimulation test	
Secondary hypothyroidism	History and physical examination. Clinical differentiation from primary form difficult	T_4, RT_3U, serum TSH or TSH stimulation test, thyroid antibodies, other pituitary hormones	TRH stimulation test if hypothalamic origin suspected (pituitary dwarfism).
Thyroid aplasia or ectopism	Neonatal hypothyroidism, absence of goiter. In case of slight suspicion: T_4, RT_3U	T_4, RT_3U, ^{131}I uptake and scan	TSH stimulation test or serum TSH
Familial defect in hormone biosynthesis	Goiter and hypothyroidism in newborn. Affected sibling	T_4, RT_3U, ^{131}I uptake. Perchlorate discharge test. Deiodinase test. Biochemical examination of excised gland	
Euthyroid sporadic goiter	History and physical examination	T_4, RT_3U (^{131}I uptake and scan). Surgery if malignancy suspected	Scan if there is slightest doubt of toxic adenoma
Endemic goiter	History and physical examination	T_4, RT_3U, ^{131}I uptake	Urinary iodine excretion
Chronic lymphocytic thyroiditis	Clinical evaluation. Antibodies to thyroid tissue	Patient may be hypo-, eu-, or hyperthyroid. Therefore: T_4, RT_3U (^{131}I uptake, scan)	Biopsy
Acute or subacute granulomatous thyroiditis	Clinical evaluation: pain, fatigue. Elevated erythrocyte sedimentation rate	Thyroid antibodies (absent) T_4, RT_3U, ^{131}I uptake, scan	Biopsy

in some studies and a low rating in others. The *ankle jerk* appears to be useful for the diagnosis of hypothyroidism, but not very useful in hyperthyroidism. The plasma *cholesterol*, as mentioned earlier, proved to be practically useless as a thyroid function test.

Finally it is noteworthy that in FRAGU's (1972) study the *clinical impression* was unsurpassed by any single laboratory test for the diagnosis of hyperthyroidism, which may be a comfort to the many doctors who have to practice without the luxury of ready access to an endocrine laboratory.

Table 27 gives a summary of the various tests we find most useful for a number of common thyroid disorders.

References

The Thyroid Gland

Basic Texts

BASTÉNIE, P.A., ERMANS, A.M.: Thyroiditis and thyroid function. Oxford: Pergamon 1972.

HEDINGER, CHR. (ed.): Thyroid cancer. UICC Monograph Series, vol. 12. Berlin-Heidelberg-New York: Springer 1969.

MEANS, J.H., DEGROOT, L.J., STANBURY, J.B.: The thyroid and its diseases, 3rd. ed. New York-Toronto-London: McGraw-Hill Book Co. 1963.

PITT-RIVERS, R., TROTTER, W.R.: The thyroid gland. London: Butterworths 1964.

STANBURY, J.B., KROC, R.L. (ed.): Human development and the thyroid gland. Relation to endemic cretinism. New York: Plenum Press 1972.

Symposium on Graves' disease. Mayo Clin. Proc. **47**, 801 (1972).

WAYNE, E.J., KOUTRAS, D.A., ALEXANDER, W.D.: Clinical aspects of iodine metabolism. Oxford: Blackwell 1964.

WERNER, S.C., INGBAR, S.H.: The thyroid: A fundamental and clinical text, 3rd ed. New York: Harper & Row 1972.

Embryology and Anatomy

ALLEN, B.M.: The influence of the thyroid gland and hypophysis upon growth and development of amphibian larvae. Quart. Rev. Biol. **4**, 325 (1929).

— The endocrine control of amphibian metamorphosis. Biol. Rev. **13**, 1 (1938).

BARGMANN, W.: Die Schilddrüse. Handbuch der mikroskopischen Anatomie des Menschen, Bd. VI/2. 1939.

EKHOLM, R., SMEDS, S.: On dense bodies and droplets in the follicular cells of the guinea pig thyroid. J. Ultrastruct. Res. **16**, 71 (1966).

ETKIN, W.: A thyroid field effect in the tadpole. J. exp. Zool. **82**, 463 (1939).

FUGO, N.W.: Effects of hypophysectomy in the chick embryo. J. exp. Zool. **85**, 271 (1940).

GOLDSMITH: Phylogeny of the thyroid, descriptive and experimental. Ann. N.Y. Acad. Sci. **50**, 283 (1949).

GONZALES, F.: Functional differentiation of embryonic chick thyroid in roller tube cultures. Exp. Cell Res. **10**, 181—187 (1956).

GORBMANN, A.: Phylogenetic and developmental aspects of the thyroid. Brookhaven Symp. in Biol. The thyroid. Upton, N.Y.: Brookhaven National Laboratory 1955.

GREENBERG, A.H., CZERNICHOW, P., REBA, R.C., TYSON, J., BLIZZARD, R.M.: Observations on the maturation of thyroid function in early fetal life. J. clin. Invest. **49**, 1790 (1970).

GRUMBACH, M.M., WERNER, S.C.: Transfer of thyroid hormone across the human placenta at term. J. clin. Endocr. **16**, 1392 (1956).

HILFER, S.R.: The stability of embryonic chick thyroid cells *in vitro*. Develop. Biol. **4**, 1—21 (1963).

HODGES, R.E., EVANS, T.C., BRADBURY, J.T., KEETTEL, W.C.: The accumulation of radioactive iodine by human fetal thyroids. J. clin. Endocr. **15**, 661 (1955).

JOST, A.: Hormonal factors in the development of the fetus. Cold. Spr. Harb. Symp. quant. Biol. A, 167 (1954).

— MOREL, F.F., MAROIS, M.: Données préliminaires sur la fixation de radio-iode I^{131} par la thyroide fétale du lapin. C.R. Soc. Biol. (Paris) **143**, 142 (1949).

KERR, T.: On the histology of the developing pituitary in the frog (Rana t. temporaria) and in the toad (Bufo bufo). Proc. zool. Soc. Lond. B **109**, 167 (1939).

MARTINDALE, F.M.: Initiation and early development of thyrotropic function in the incubating chick. Anat. Rec. **79**, 373 (1941).

NADLER, N.J., YOUNG, B.A., LEBLOND, C.P., MITMAKER, B.: Elaboration of thyroglobulin in the thyroid follicle. Endocrinology **74**, 333—354 (1964).

RANKIN, R.M.: Changes in the content of iodine compounds and in the histological structure of the thyroid gland of the pig during fetal life. Anat. Rec. **80**, 123 (1941).

ROBERTIS, E. DE: Cytological and cytochemical bases of thyroid function. Ann. N.Y. Acad. Sci. **50**, 317 (1949).

SELJELID, R.: Endocytosis in thyroid follicle cells: I. Structure and significance of different types of single membrane-limited vacuoles and bodies. J. Ultrastruct. Res. **17**, 195 (1967).

— Endocytosis in thyroid follicle cells: II. A Microinjection study of the origin of colloid droplets. J. Ultrastruct. Res. **17**, 401 (1967).

— Endocytosis in thyroid follicle cells: V. On the redistribution of cytosomes following stimulation with thyrotropic hormone. J. Ultrastruct. Res. **18**, 479 (1967).

STEIN, O., GROSS, J.: Metabolism of ^{125}I in the thyroid gland studied with electron microscopic autoradiography. Endocrinology **75**, 787—798 (1964).

TONUTTI, E.: Normale Anatomie der endokrinen Drüsen und endokrine Regulation. In Lehrbuch der speziellen pathologischen Anatomie von E. KAUFMANN, herausgeg. von M. STAEMMLER, 11th and 12th ed., Bd. 1, part 2, p. 1285. Berlin: W. de Gruyter & Co. 1955.

WEGELIN, C.: Schilddrüse, S. 1. In: F. HENKE u. O. LUBARSCH, Handbuch der speziellen pathologischen Anatomie, Bd. 8. Drüsen mit innerer Sekretion. Berlin: Springer 1926.

WOLFF, J., CHAIKOFF, J.L., NICHOLS, C.W. JR.: The accumulation of thyroxine-like and other iodine compounds in the fetal bovine thyroid. Endocrinology **44**, 510 (1949).

WOLLMANN, S.H., ZWILLING, E.: Radio-iodine metabolism in the chick embryo. Endocrinology **52**, 526 (1953).

Biochemistry and Physiology

Reviews

DUMONT, J.E.: The action of thyrotropin on thyroid metabolism. Vitam. and Horm. **29**, 287 (1971).

FELLENBERG, TH. VON: Das Vorkommen, der Kreislauf und der Stoffwechsel des Jods. Ergebn. Physiol. **25**, 176 (1926).

GALTON, V.A.: The physiological role of thyroid hormone metabolism. In: JAMES: Recent advances in endocrinology. 8th ed. London: Churchill 1968.

HARINGTON, C.R.: Newer Knowledge of the biochemistry of the thyroid gland. J. chem. Soc. **1944**, 193.

HILLMANN, G.: Biosynthese und Stoffwechselwirkungen der Schilddrüsenhormone. Stuttgart: Thieme 1961.

LARSEN, P. R.: Triiodothyronine: Review of recent studies of its physiology and pathophysiology in man. Metabolism **21**, 1073 (1972).

PITT-RIVERS, R., TATA, J. R.: The thyroid hormones. London: Pergamon Press 1959.

ROBBINS, J., RALL, J. E.: The iodine-containing hormones. In: GRAY, C. H., and A. L. BACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

Thyroid Hormone Chemistry and Analogues

BANSI, H. W.: Schilddrüsenhormonanaloge und -metaboliten unter besonderer Berücksichtigung ihrer klinischen Anwendung. Ergebn. inn. Med. Kinderheilk. **18**, 196 (1962).

BARKER, S. B.: Chemistry, cellular and subcellular effects of thyroid hormones. In: The Thyroid S. C. WERNER and S. H. INGBAR, (eds.), p. 79. New York: Harper and Row 1971.

BENUA, R. S., LEEPER, R. D., KUMAOKA, S., RAWSON, R. W.: Metabolic effects of thyroxine analogues in human myxedema. Ann. N. Y. Acad. Sci. **86**, 563 (1960).

CAMERMAN, N., CAMERMAN, A.: Three-dimensional structure of L-thyroxine. Proc. nat. Acad. Sci. (Wash.) **69**, 2130 (1972).

— — Triiodothyronine: The 3′-iodine is proximal to the α-ring in crystal structure conformation. Science **175**, 764 (1972).

JORGENSEN, E. C., BERTEAU, P. E.: Thyroxine analogs. 21. o-and m-L-thyroxine and related compounds. J. medicinal Chem. **14**, 1199 (1971).

MONEY, W. L., KUMAOKA, S., RAWSON, R. W., KROC, R. L.: Comparative effects of thyroxine analogues in experimental animals. Ann. N. Y. Acad. Sci. **86**, 512 (1960).

RHODES, B. A.: The circulating iodotyrosines. Acta endocr. (Kbh.), Suppl. **127**, to Bd. 57 (1968).

ROBBINS, J., WOLFF, J., RALL, J. E.: Iodoproteins in normal and abnormal human thyroid tissue and in normal sheep thyroid. Endocrinology **64**, 37 (1959).

Thyroid Hormone Biosynthesis and Release

ALEXANDER, N. M., CORCORAN, B. J.: Reversible dissociation of thyroid iodide peroxidase into apoenzyme and prosthetic group. J. biol. Chem. **237**, 243 (1962).

ALEXANDER, W. D., KOUTRAS, D. A., CROOKS, J., BUCHANAN, W. W., MACDONALD, E. M., RICHMOND, M. H., WAYNE, E. J.: Quantitative studies of iodine metabolism in thyroid disease. Quart. J. Med., N. S. **31**, 281 (1962).

ARIMA, T., SPIRO, M. J., SPIRO, R. G.: Studies on the carbohydrate units of thyroglobulin. Evaluation of their microheterogeneity in the human and calf proteins. J. biol. Chem. **247**, 1825 (1972).

— SPIRO, R. G.: Studies on the carbohydrate units of thyroglobulin. Structure of the mannose *N*-acetylglucosamine unit (unit A) of the human and calf proteins. J. biol. Chem. **247**, 1836 (1972).

BENABDELJLIL, C., MICHEL-BÉCHET, M., LISSITZKY, S.: Isolation and iodinating ability of apical poles of sheep thyroid epithelial cells. Biochem. biophys. Res. Commun. **27**, 74 (1967).

BLASI, F., FRAGOMELE, F., COVELLI, I.: Enzymic pathway for thyroxine synthesis through p-hydroxy-3,5-diiodophenylpyruvic acid. Endocrinology **85**, 542 (1969).

BROWN-GRANT, K.: Extrathyroidal iodide concentrating mechanisms. Physiol. Rev. **41**, 189 (1961).

BÜRGI, H., ANDERSEN, M.-C., SCHWANDER, J., KOHLER, H., STUDER, H.: Secretion of thyroxine and non-thyroxine iodine by the normal human thyroid gland. Influence of carbimazole and pharmacological doses of iodine. Europ. J. clin. Invest. **3**, 142 (1973).

COVAL, M. L., TAUROG, A.: Purification and iodinating activity of hog thyroid peroxidase. J. biol. Chem. **242**, 5510 (1967).

CROMBRUGGHE, B. DE, EDELHOCH, H.: The properties of thyroglobulin. XIV. The structure of reoxidized thyroglobulin. Biochem. J. **5**, 2238 (1966).

DANNER, D. J., MORRISON, M.: Isolation of the thyroid peroxidase complex. Biochim. biophys. Acta (Amst.) **235**, 44 (1971).

DE GROOT, L. J.: Current views on formation of thyroid hormones. New Engl. J. Med. **272**, 243 (1965).

DEISS, W. P., PEAKE, R. L.: The mechanism of thyroid hormone secretion. Ann. intern. Med. **69**, 881 (1968).

DUMAS, P., MAZIÈRE, B., AUTISSIER, N., MICHEL, R.: Specificité de l'iodotyrosine désiodase des microsomes thyroïdiens et hépatiques. Biochim. biophys. Acta (Amst.) **293**, 36 (1973).

EDELHOCH, H.: The structure of thyroglobulin and its role in iodination. Recent Progr. Hormones Res. **21**, 1 (1965).

GREGERMAN, R. I., GAFFNEY, G. W., SHOCK, N. W.: Thyroxine turnover in euthyroid man with special reference to changes with age. J. clin. Invest. **41**, 2065 (1962).

HAIBACH, H.: Free iodothyronines in the rat thyroid gland. Endocrinology **88**, 149 (1971).

HALMI, N. S., PITT-RIVER, R.: The iodide pools of the rat thyroid. Endocrinology **70**, 660 (1962).

HARINGTON, C. R.: Newer knowledge of the biochemistry of the thyroid gland. J. chem. Soc. **1944**, 193.

HUBER, D., STUDER, H.: "Altes" und "junges" Jod in der Schilddrüse: Hinweis auf die funktionelle Heterogenität der menschlichen Schilddrüse. Helv. med. Acta, Suppl. **47**, 117 (1967).

KOJ, TOI, SALVATORE, G., CAHNMANN, H. J.: Model reactions for the biosynthesis of thyroxine. X. Non-enzymic formation of thyroxine and 3,3′,5′-Triiodthyronine residues in thyroglobulin. Biochim. biophys. Acta (Amst.) **97**, 523 (1965).

LAMAS, L., DORRIS, M. C., TAUROG, A.: Evidence for a catalytic role for thyroid peroxidase in the conversion of diiodotyrosine to thyroxine. Endocrinology **90**, 1417 (1972).

LISSITZKY, S.: Biosynthèse des hormones thyroïdiennes. Expos. ann. Biochim. méd. **25**, 1 (1964).

— ROLLAND, M., REYNAUD, J., SAVARY, J., LASRY, S.: Structure sous-unitaire de la thyroglobuline de mouton. Europ. J. Biochem. **4**, 464 (1968).

MAYBERRY, W. E., RALL, J. E., BERTOLI, D.: Kinetics of iodination. J. Amer. chem. Soc. **86**, 5302 (1964).

MELTZER, R. I., STANABACK, R. J.: Reaction of 4-hydroxy-3,5 diiodophenylpyruvic acid with 3,5-diiodotyrosine. J. organ. Chem. **26**, 1976 (1961).

MUTZENBECHER, P. VON: Über die Bildung von Thyroxin aus Dijodtyrosin. Hoppe-Seylers Z. physiol. Chem. **261**, 253 (1939).

NADLER, N. J., LEBLOND, C. P.: The site and rate of the formation of thyroid hormone. In: The thyroid. Brookhaven Symp. Biol. **7**, 40 (1955).

NAGATAKI, S., INGBAR, S. H.: Demonstration of a second thyroidal iodide pool in rat thyroid glands by double isotope labeling. Endocrinology **73**, 479 (1963).

— SHIZUME, K., NAKAO, K.: Thyroid function in chronic excessive iodide ingestion: release of inorganic iodide from the thyroid gland *in vitro* in response to chronic iodide administration. Endocrinology **85**, 899 (1969).

NELSON, J. C., WEISS, R. M., LEWIS, J. E., WILCOX, R. B.: Radioimmunoassay of diiodotyrosine in serum. Proc. 48th Annual Meeting American Thyroid Association, p. 17 (1972).

NICOLOFF, J. T., LOW, J. C., DUSSAULT, J. H., FISHER, D. A.: Simultaneous measurements of thyroxine and triiodothyronine peripheral turnover kinetics in man. J. clin. Invest. **51**, 473 (1972).

NUNEZ, J., MAUCHAMP, J., MACCHIA, V., ROCHE, J.: Biosynthèse *in vitro* d'hormones doublement marquées dans des coupes de corps thyroïde. II. Biosynthèse d'une préthyroglobuline non iodée. Biochim. biophys. Acta (Amst.) **107**, 247 (1965).
— POMMIER, J.: Iodation des protéines par voie enzymatique. 3. Complexe intermédiaire enzyme-protéine et mécanisme de la réaction. Europ. J. Biochem. **7**, 286 (1969).
ODDIE, T. H., FISHER, D. A., MCCONAHEY, W. M., THOMPSON, C. S.: Iodine intake in the United States: A reassessment. J. clin. Endocr. **30**, 659 (1970).
OGAWARA, H., CAHNMANN, H. J.: Nonenzymic synthesis of iodothyronine residues in thyroglobulin. Biochim. biophys. Acta (Amst.) **257**, 328 (1972).
OTTEN, J., JONKHEER, M., DUMONT, J. E.: Thyroid albumin. II. *In vitro* synthesis of a thyroid albumin by normal human thyroid tissue. J. clin. Endocr. **32**, 18 (1971).
PITTMAN, J. A., DAILEY, G. E., BESCHI, R. J.: Changing normal values for thyroidal radioiodine uptake. New Engl. J. Med. **280**, 1431 (1969).
PITT-RIVERS, R.: The biosynthesis of thyroid hormones. J. clin. Path. **20**, 318 (1967), Suppl.
POMMIER, J., DE PRAILAUNÉ, S., NUNEZ, J.: Peroxydase particulaire thyroïdienne. Biochimie **54**, 483 (1972).
RIVLIN, R. S., ASPER, S. P.: Thyroxine and the thyroid hormones. Amer. J. Med. **40**, 823 (1966).
ROBBINS, J., WOLFF, J., RALL, J. E.: Iodoproteins in normal and abnormal human thyroid tissue. Endocrinology **64**, 37 (1959).
ROLLAND, M., LISSITZKY, S.: Polypeptides non-covalently associated with 19-S thyroglobulin. Biochem. biophys. Acta (Amst.) **278**, 316 (1972).
— MONTFORT, M. F., VALENTA, L., LISSITZKY, S.: Iodoamino-acid composition of the thyroglobulin of normal and diseased thyroid glands. Clin. chim. Acta **39**, 95 (1972).
— ROQUES, M., TORRESANI, J., SIMON, C., BOUCHILLON, S.: Iodination and biosynthesis of rat thyroglobulin. Biochem. biophys. Res. Commun. **16**, 249 (1964).
ROSENBERG, I. N., ATHANS, J. C., ISAACS, G. H.: Studies on thyroid iodine metabolism. Recent Progr. Hormone Res. **21**, 33 (1965).
SEED, R. W., GOLDBERG, I. H.: Biosynthesis of thyroglobulin. II. Role of subunits, iodination and ribonucleic acid synthesis. J. biol. Chem. **240**, 764 (1965).
SPIRO, M. J.: Studies on the protein portion of thyroglobulin. Amino acid compositions and terminal amino acids of several thyroglobulins. J. biol. Chem. **245**, 5820 (1970).
SPIRO, R. G.: Glycoproteins: Biochemistry, biology, and role in disease. New Engl. J. Med. **281**, 991 (1969).
— SPIRO, M. J.: Glycoprotein biosynthesis. Studies of thyroglobulin J. biol. Chem. **241**, 1271 (1966).
TAUROG, A.: The biosynthesis of thyroxine. Proc. Mayo Clinic **39**, 569 (1964).
— Thyroid peroxidase and thyroxine biosynthesis. Recent Progr. Hormone Res. **26**, 189 (1970).
TOICHIRO, H., MORRISON, M.: The isolation and purification of thyroid peroxidase. J. biol. Chem. **242**, 2828 (1967).
TORRIGIANI, G., DONIACH, D., ROITT, I. M.: Serum thyroglobulin levels in healthy subjects and in patients with thyroid disease. J. clin. Endocr. **29**, 305 (1969).
VAN HERLE, A. J., ULLER, R. P., MATTEWS, N. L., BROWN, J.: Radioimmunoassay for measurement of thyroglobulin in human serum. J. clin. Invest. **52**, 1320 (1973).
VECCHIO, G., CLAAR, G. M., SALVATORE, G.: Biosynthesis of thyroid iodoproteins *in vivo* and in tissue slices. J. biol. Chem. **247**, 4908 (1972).
WALSER, M., RAHILL, W. J.: Renal tubular reabsorption of iodide as compared with chloride. J. clin. Invest. **44**, 1371 (1965).
WARTOWSKY, L., INGBAR, S. H.: Estimation of the rate of release of non-thyroxine iodine from the thyroid gland of normal subjects and patients with thyrotoxicosis. J. clin. Endocr. **33**, 488 (1971).
WEINER, H., MASUI, H., RADICHEVICH, I., WERNER, S. C.: Materials indistinguishable from iodotyrosines in normal human serum and human serum albumin. J. clin. Invest. **46**, 1264 (1967).
WILLIAMS, J. A.: Cyclic AMP formation and thyroid secretion by incubated mouse thyroid lobes. Endocrinology, **91**, 1411 (1972).
— WOLFF, J.: Thyroid secretion in vitro. Multiple actions of agents affecting secretion. Endocrinology **88**, 206 (1971).
WOLFF, J.: Transport of iodide and other anions in the thyroid gland. Physiol. Rev. **44**, 45 (1964).

Thyroid Hormones in Plasma

BAYLEY, T. A., HIGGINS, H. D., ROW, V. V., JAMES, W., VIDAL, R.: The metabolic significance of thyroxine-binding globulin: studies in a family with decreased thyroxine-binding globulin. Acta endocr. (Kbh.) **61**, 137 (1969).
BEIERWALTES, W. H., ROBBINS, J.: Familial increase in the thyroxine-binding sites in serum alpha globulin. J. clin. Invest. **38**, 1683 (1959).
BEISEL, W. R., ZAINAL, H., HANE, S., DI RAIMONDO, V. C., FORSHAM, P. H.: Low thyroidal iodine uptake with euthyroidism associated with deficient thyroxine-binding globulin but normal cortisol binding. J. clin. Endocr. **22**, 1165 (1962).
BÉRAUD, T.: Etudes biochimiques des hormones thyroïdiennes en pathologie humaine. Acta endocr. (Kbh.), Suppl. **55** to Bd. 35 (1960).
BRAVERMAN, L. E., DAWBER, N. A., INGBAR, S. H.: Observations concerning the binding of thyroid hormones in sera of normal subjects of varying ages. J. clin. Invest. **45**, 1273 (1966).
— FOSTER, A. E., INGBAR, S. H.: Sex related differences in the binding in serum of thyroid hormones. J. clin. Endocr. **27**, 227 (1967).
CLARK, F.: The estimation of thyroid hormone binding by plasma proteins and of unbound levels of thyroxine in plasma. J. clin. Path. **20**, 344 (1967), Suppl.
DAVIS, P. J., GREGERMAN, R. I.: Separation of thyroxine-binding proteins in human serum at pH 7.4. II. Effect of pH and temperature on the binding capacities of thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA). J. clin. Endocr. **33**, 699 (1971).
— HANDWERGER, B. S., GREGERMAN, R. I.: Thyroid hormone binding by human serum prealbumin (TBPA). Electrophoretic studies of triiodothyronine—TBPA interaction. J. clin. Invest. **51**, 515 (1972).
DE RUBERTIS, F. R., WOEBER, K. A.: Evidence for enhanced cellular uptake and binding of thyroxine *in vivo* during acute infection with diplococcus pneumoniae. J. clin. Invest. **51**, 788 (1972).
FERRIER, P. E., LEMARCHAND-BÉRAUD, T.: Variations of thyroid hormone transport in the blood of normal children and of children with mongolism. Acta endocr. (Kbh.) **48**, 547 (1965).
FLORSHEIM, W. H., DOWLING, J. T., MEISTER, L., BODFISH, R. E.: Familial elevation of serum thyroxine-binding capacity. J. clin. Endocr. **22**, 735 (1962).
HEINONEN, O. P., LAMBERG, B.-A., VIRTANO, J.: Inherited decrease of the binding capacity of thyroxine-binding globulin. Acta endocr. (Kbh.) **64**, 171 (1970).
HODGSON, S. F., WAHNER, H. W.: Hereditary increased thyroxine-binding globulin capacity. Mayo Clin. Proc. **47**, 720 (1972).
HOLLANDER, C. S., SCOTT, R. L., TSCHUDY, D. P., PERLROTH, M., WAXMAN, A., STERLING, K.: Increased protein-bound iodine and thyroxine-binding globulin in acute intermittent porphyria. New Engl. J. Med. **277**, 995 (1967).
HORN, K., RUHL, T., SCRIBA, P. C.: Semiautomatic method for the separation and determination of total triiodo-

thyronine and thyroxine in serum. Z. klin. Chem. **10**, 99 (1972).

INGBAR, S. H., FREINKEL, N.: Regulation of peripheral metabolism of thyroid hormones. Recent Progr. Hormone Res. **16**, 353 (1960).

KRAEMER, E., WISWILL, J. G.: Familiar thyroxine-binding globuline deficiency. Metabolism **17**, 260 (1968).

LARSEN, P. R., ATKINSON, A. J., WELLMAN, H. N., GOLDSMITH, R. E.: The effect of diphenylhydantoin on thyroxine metabolism in man. J. clin. Invest. **49**, 1266 (1970).

— Salicyclate-induced increases in free triiodothyronine in human serum. Evidence of inhibition of triiodothyronine binding to thyroxine-binding globulin and thyroxine-binding prealbumin. J. clin. Invest. **51**, 1125 (1972).

LEMARCHAND-BÉRAUD, T., VANOTTI, A.: Transport of thyroid hormones in human pathology. Europ. Rev. Endocr. **1**, 163 (1964).

LIEBLICH, J., UTIGER, R. D.: Triiodothyronine radioimmunoassay. J. clin. Invest. **51**, 157 (1972).

MARSHALL, J. S., LEVY, R. P., STEINBERG, A. G.: Human thyroxine-binding globulin deficiency. A genetic study. New Engl. J. Med. **274**, 1469 (1966).

MCCONNON, J., ROW, V. V., VOLPÉ, R.: Simultaneous comparative studies of thyroxine and triiodothyronine distribution and disposal rates. J. Endocr. **51**, 17 (1971).

MITSUMA, T., NIHEI, N., GERSHENGORN, M. C., HOLLANDER, C. S.: Serum triiodothyronine: measurements in human serum by radioimmunoassay with corroboration by gas liquid chromatography. J. clin. Invest. **50**, 2679 (1971).

NAUMAN, J. A., NAUMAN, A., WERNER, S. C.: Total and free triiodothyronine in human serum. J. clin. Invest. **46**, 1346 (1967).

NICOLOFF, J. T., DOWLING, J. T., PATTON, D. D.: Inheritance of decreased thyroxine-binding by the thyroxine-binding globulin. J. clin. Endocr. **24**, 294 (1964).

— LOW, J. C., DUSSAULT, J. H., FISHER, D. A.: Simultaneous measurements of thyroxine and triiodothyronine peripheral turnover kinetics in man. J. clin. Invest. **51**, 473 (1972).

NIKOLAI, T. F., SEAL, U. S.: X-chromosome linked decrease in thyroxine-binding globulin activity. J. clin. Endocr. **26**, 835 (1966).

NUSYNOWITZ, M. L., CLARK, R. F., STRADER, W. J., ESTRIN, H. M., SEAL, U. S.: Thyroxine-binding globulin deficiency in three families and total deficiency in one normal woman. Amer. J. Med. **50**, 458 (1971).

ODDIE, T. H., FISHER, D. A., DUSSAULT, J. H., THOMPSON, C. S.: Triiodothyronine turnover in euthyroid subjects. J. clin. Endocr. **33**, 653 (1971).

— MEADE, H., FISHER, D. A.: An analysis of published data on thyroxine turnover in human subjects. J. clin. Endocr. **26**, 425 (1966).

OPPENHEIMER, J. H.: Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. New Engl. J. Med. **278**, 1153 (1968).

OSORIO, C.: Carriage of circulating thyroid hormones and the estimation of total plasma hormone levels. J. clin. Path. **20**, 335 (1967), Suppl.

— PENSKY, J.: Studies on human thyroxine-binding globulin (TBG). Purification of TBG and immunologic studies on the relationship between TBG from normal persons and those with TBG "deficiency". J. clin. Invest. **48**, 508 (1969).

REFETOFF, S., ROBIN, N. I., ALPER, C. A.: Study of four new kindreds with inherited thyroxine-binding globulin abnormalities. Possible mutations of a single gene locus. J. clin. Invest. **51**, 848 (1972).

ROBBINS, J., RALL, J. E.: The iodine-containing hormones. In: GRAY, C. H., and A. L. BACHARACH. Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

SCAZZIGA, B. R.: Fonction thyroïdienne et métabolisme iodé dans la sénescence. Schweiz. med. Wschr. **96**, 1558 (1966).

SHANE, S. R., SEAL, U. S., JONES, J. E.: X-chromosome linked inheritance of elevated thyroxine binding globulin in association with goiter. J. clin. Endocr. **32**, 587 (1971).

STERLING, K.: Thyroxine in blood. Proc. Mayo Clin. **39**, 586 (1964).

— BELLABARBA, D., NEWMAN, E. S., BRENNER, M. A.: Determination of triiodothyronine concentration in human serum. J. clin. Invest. **48**, 1150 (1969).

— HAMADA, S., TAKEMURA, Y., BRENNER, M. A., NEWMAN, E. S., INADA, M.: Preparation and properties of thyroxine-binding globulin. J. clin. Invest. **50**, 1758 (1971).

— SURKS, M. I., SCHWARTZ, H. L.: The metabolic significance of exchangable cellular thyroxine. Recent Progr. Hormone Res. **25**, 381 (1969).

TANAKA, S., STARR, P.: A euthyroid man without thyroxine binding globulin. J. clin. Endocr. **19**, 485 (1959).

TORKINGTON, P., HARRISON, R. J., MACLAGAN, N. F., BURSTON, D.: Familial thyroxine-binding globulin deficiency. Brit. med. J. **3**, 27 (1970).

WOEBER, K. A., INGBAR, S. H.: The contribution of thyroxine-binding prealbumin to the binding of thyroxine in human serum, as determined by immunoadsorption. J. clin. Invest. **47**, 1710 (1968).

— SOBEL, R. J., INGBAR, S. H., STERLING, K.: The peripheral metabolism of triiodothyronine in normal subjects and in patients with hyperthyroidism. J. clin. Invest. **49**, 643 (1970).

VANOTTI, A., BÉRAUD, T.: Functional relationships between the liver, the thyroxine-binding protein of serum and the thyroid. J. clin. Endocr. **19**, 466 (1959).

Thyroid Hormone Metabolism

ANBAR, M., GUTTMANN, S., RODAN, G., STEIN, J. A.: The determination of the rate of deiodination of radioactive thyroxine in human subjects. J. clin. Invest. **44**, 1986 (1965).

BURKE, C. W., SHAKESPEAR, R. A., FRASER, T. R.: Measurement of thyroxine and triiodothyronine in human urine. Lancet **1972 II**, 1177.

BRAVERMAN, L. E., INGBAR, S. H., STERLING, K.: Conversion of thyroxine to triiodothyronine in athyrotic human subjects. J. clin. Invest. **49**, 855 (1970).

CHAN, U., BESSER, G. M., LANDON, J., EKINS, R. P.: Urinary triiodothyronine excretion as index of thyroid function. Lancet **1972 II**, 253.

CHOPRA, I. J., FISHER., D. A., SOLOMON, D. H., BEALL, G. N.: Thyroxine and triiodothyronine in the human thyroid. J. clin. Endocr. **36**, 311 (1973).

DOWLING, J. T., APPLETON, W. E., MUSA, B. U.: Direct measurement of hepatic thyroxine flux in normal man. J. clin. Endocr. **28**, 1403 (1968).

GREGERMAN, R. I., GAFFNEY, G. W., STROCK, N. W.: Thyroxine turnover in euthyroid man with special reference to changes with age. J. clin. Invest. **41**, 2065 (1962).

INGBAR, S. H., FREINKEL, N.: Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis. J. clin. Invest. **34**, 808 (1955).

— — Regulation of the peripheral metabolism of the thyroid hormones. Recent. Progr. Hormone Res. **6**, 353 (1960).

IRVINE, C. H.: Effect of exercise on thyroxine degradation in athletes and non-athletes. J. clin. Endocr. **28**, 942 (1968).

KOZYREFF, V., SURKS, M. I., OPPENHEIMER, J. H.: Demonstration of membrane-linked iodoprotein in hepatic microsomes following metabolism of thyroid hormones. Endocrinology **86**, 781 (1970).

OPPENHEIMER, J. H., BERNSTEIN, G., SURKS, M. I.: Increased thyroxine turnover and thyroidal function following stimulation of hepatocellular binding of thyroxine by phenobarbital. J. clin. Invest. **47**, 1399 (1968).

PITTMAN, C.S., BUCK, M.W., CHAMBERS, J.B.: Urinary metabolites of ^{14}C-labeled thyroxine in man. J. clin. Invest. **51**, 1759 (1972).
— CHAMBERS, J.B., READ, V.H.: The extrathyroidal conversion rate of thyroxine to triiodothyronine in normal man. J. clin. Invest. **50**, 1187 (1971).
— READ, V.H., CHAMBERS, J.B., NAKAFUJI, H.: The integrity of the ether linkage during thyroxine metabolism in man. J. clin. Invest. **49**, 373 (1970).
SCHWARTZ, H.L., SURKS, M.I., OPPENHEIMER, J.H.: Quantitation of extrahthyroidal conversion of L-thyroxine to 3,5,3'-triiodothyronine in the rat. J. clin. Invest. **50**, 1124 (1971).
SOFFER, R.L., HECHTMAN, P., SANAGE, M.: L-Triiodothyronine aminotransferase. J. biol. Chem. **248**, 1224 (1973).
STERLING, K., BRENNER, M.A., NEWMAN, E.S.: Conversion of thyroxine to triiodothyronine in normal human subjects. Science **169**, 1099 (1970).
SURKS, M.I., SCHADLOW, A.R., STOCK, J.M., OPPENHEIMER, J.H.: Determination of iodothyronine absorption and conversion of *L*-thyroxine (T_4) to L-triiodothyronine (T_3) using turnover techniques. J. clin. Invest. **52**, 805 (1973).
— SCHWARTZ, H.L., OPPENHEIMER, J.H.: Tissue iodoprotein formation during the peripheral metabolism of the thyroid hormones. J. clin. Invest. **48**, 2168 (1969).
TATA, J.R.: Intracellular and extracellular mechanisms for the utilization and action of thyroid hormones. Recent Progr. Hormone Res. **18**, 221 (1962).

Mechanism of Action of Thyroid Hormones

ASKONAS, B.A.: Effect of thyroxine on creatine-phosphokinase activity. Nature (Lond.) **167**, 933 (1959).
BARKER, S.B., KLITGAARD, H.M.: Metabolism of tissues excised from thyroxine injected rats. Amer. J. Physiol. **170**, 81 (1952).
BRONK, J.A., BRONK, M.S.: The influence of thyroxine on oxidative phosphorylation in mitochondria from thyroidectomized rats. J. biol. Chem. **237**, 897 (1962).
BUCHANAN, J.L., PRIMACK, M.P., TAPLEY, D.F.: Effect of inhibition of mitochondrial protein synthesis *in vitro* upon thyroxine stimulation of oxygen consumption. Endocrinology **89**, 534 (1971).
BUCCINO, R.A., SPANN, J.F., JR., POOL, P.E., SONNENBLICK, E.H., BRAUNWALD, E.: Influence of the thyroid state on the intrinsic contractile properties and energy stores of the myocardium. J. clin. Invest. **46**, 1669 (1967).
BUTTERFIELD, W.J.H., WHICHELOW, M.J.: Are thyroid hormones diabetogenic? A study of peripheral glucose, metabolism during glucose infusions in normal subjects and hyperthyroid patients before and after treatment. Metabolism, **13**, 620 (1964).
CALDWELL, A., FAIN, J.N.: Triiodothyronine stimulation of cyclic adenosine 3',5'-monophosphate accumulation in fat cells. Endocrinology **89**, 1195 (1971).
CARTER, W.J., FAAS, F.H., WYNN, J.: Thyroxine stimulation of protein synthesis *in vitro* in the absence of mitochondria. J. biol. Chem. **246**, 4973 (1971).
CHALLONER, D.D., ALLEN, D.O.: An *in vitro* effect of triiodothyronine on lipolysis, cyclic AMP-C^{14} accumulation and oxygen consumption in isolated fat cells. Metabolism **19**, 480 (1970).
COHEN, P.P.: Biochemical differentiation during amphibian metamorphosis. Science **168**, 533 (1970).
CRISPELL, K.R., PARSON, W., HOLLIFIELD, G.: Study of the rate of protein synthesis before and during the administration of L-triiodothyronine to patients with myxedema and healthy volunteers using N-15-glycine. J. clin. Invest. **35**, 164 (1956).
DAYTON, S., DAYTON, J., DRIMMER, F., KENDALL, F.E.: Rates of acetate turnover and lipid synthesis in normal hypothyroid and hyperthyroid rats. Amer. J. Physiol. **199**, 71 (1960).
DEGROOT, W.J., LEONARD, J.J.: Hyperthyroidism as a high cardiac output state. Amer. Heart J. **79**, 265 (1970).
EPSTEIN, F.H.: Bone and mineral metabolism in hyperthyroidism. Ann. intern. Med. **68**, 490 (1968).
ERNSTER, L., IKKOS, D., LUFT, R.: Enzymatic activities of human skeletal muscle mitochondria: a tool in clinical metabolic research. Nature (Lond.) **184**, 1851 (1959).
FUHR, J.E., MEDICI, P.: An *in vitro* effect of thyroid hormone upon bone marrow synthesis of hemoglobin. FEBS Letter **11**, 20 (1970).
GALTON, V.A., INGBAR, S.H.: Observation on the relation between the action and the degradation of thyroid hormones as indicated by studies in the tadpole and the frog. Endocrinology **70**, 622 (1962).
GELBER, S., CAMPBELL, P.L., DEIBLER, G.E., SOKOLOFF, L.: Effects of L-thyroxine on amino acid incorporation into protein in mature and immature rat brain. J. Neurochem. **11**, 221 (1964).
GREIF, R.L.: Thyroxine and the release of protein from rat liver mitochondria. Endocrinology **70**, 511 (1962).
GRISWOLD, M.D., COHEN, P.P.: Alteration of deoxyribonucleic acid-dependent ribonucleic acid polymerase activities in amphibian liver nuclei during thyroxine-induced metamorphosis. J. biol. Chem. **247**, 353 (1972).
— FISHER, M.S., COHEN, P.P.: Temperature dependent intracellular distribution of thyroxine in amphibian liver. Proc. nat. Acad. Sci. (Wash.) **69**, 1486 (1972).
HEITZMANN, R.J., HIBITT, K.G., MATHER, I.: The effects of thyroxine on hepatic gluconeogenesis and ketogenesis in dairy cows. Europ. J. Biochem. **21**, 411 (1971).
HOCH, F.L.: Thyrotoxicosis as a disease of mitochondria. New Engl. J. Med. **266**, 446 (1962).
— Biochemical actions of thyroid hormones. Physiol. Rev. **42**, 605 (1962).
— Early action of injedted L-thyroxine on mitochondrial oxidative phosphorylation. Proc. nat. Acad. Sci. (Wash.) **58**, 506 (1967).
— LIPMANN, F.: The uncoupling of respiration and phosphorylation by thyroid hormones. Proc. nat. Acad. Sci. (Wash.) **40**, 909 (1954).
— MOTTA, M.V.: Reversal of early thyroid hormone action on mitochondria by bovine serum albumin. Proc. nat. Acad. Sci. (Wash.) **59**, 118 (1968).
HOFMANN, W.W., DENYS, E.H.: Effects of thyroid hormone at the neuromuscular junction. Amer. J. Physiol. **223**, 283 (1972).
ISMAIL-BEIGI, F., EDELMAN, I.S.: The mechanism of the calorigenic action of thyroid hormone. J. gen. Physiol. **57**, 710 (1971).
JEANRENAUD, B.: Dynamic aspects of adipose tissue metabolism: a review. Metabolism **10**, 535 (1961).
KAPLAY, S.S., SANADI, D.R.: Thyroxine-induced mitochondrial protein and its effect on respiration. Arch. Biochem. **144**, 440 (1971).
KIMATA, S., TARJAN, E.M.: Effects of *in vivo* and *in vitro* administered thyroxine on substrate metabolism of isolated rabbit ventricle mitochondria. Endocrinology **89**, 378 (1971).
KRANE, S.M., BROWNELL, G.L., STANBURY, J.B.: The effect of thyroid disease on the metabolism of calcium in man. J. clin. Invest. **34**, 946 (1955).
KRISHNA, G., HYNIE, S., BRODIE, B.B.: Effects of thyroid hormones on adenyl cyclase in adipose tissue and on free fatty acid mobilization. Proc. nat. Acad. Sci. (Wash.) **59**, 884 (1968).
KUHLBÄCK, B.: Creatine and creatinine metabolism in thyrotoxicosis and hypothyroidism. Acta med. scand., Suppl. 331 (1957).
LARDY, H.A.: p. 54 in Hormonal regulation of Energy Metabolism by L.W. KINSELL, Springfield: Thomas 1957.
— MALLEY, G.F.: Metabolic effects of thyroid hormones *in vitro*. Recent. Progr. Hormone Res. **10**, 129 (1954).
LEHNINGER, A.L.: Reversal of thyroxine induced swelling of rat liver mitochondria by adenosine triphosphate. J. biol. Chem. **234**, 2187 (1959).

— Water uptake and extrusion by mitochondria in relation to oxidative phosphorylation. Phys. Rev. **42**, 467 (1962).
LEVEY, G.S.: Catecholamine sensitivity, thyroid hormone and the heart. A re-evaluation. Amer. J. Med. **50**, 413 (1971).
— EPSTEIN, S.E.: Myocardial adenyl cyclase activation by thyroid hormones and evidence for 2 adenyl cyclase systems. J. clin. Invest. **48**, 1663 (1969).
— SKELTON, C.L., EPSTEIN, S.E.: Influence of hyperthyroidism on the effects of norepinephrine on myocardial adenyl cyclase activity and contractile state. Endocrinology **85**, 1004 (1969).
LOOMIS, W.F., LIPMANN, F.: Reversible inhibition of the coupling between phosphorylation and oxidation. J. biol. Chem. **173**, 807 (1948).
MARTIUS, C.: Die Wirkungsweise des Schilddrüsenhormones. In: Hormone und ihre Wirkungsweise. Berlin-Göttingen-Heidelberg: Springer 1955.
OPPENHEIMER, J.H., KOERNER, D., SCHWARTZ, H.L., SURKS, M.I.: Specific nuclear triiodothyronine binding-sites in rat liver and kidney. J. clin. Endocr. **35**, 330 (1972).
— SHAPIRO, H.C., SCHWARTZ, H.L., SURKS, M.I.: Dissociation between thyroxine metabolism and hormonal action in phenobarbital treated rats. Endocrinology **88**, 115 (1971).
PAIK, W.K., METHENBERG, R.L., COHEN, P.P.: Biochemical studies on amphibian metamorphosis. III. Metabolism of nucleic acids and nucleotides in tadpole liver during thyroxine-induced metamorphosis. J. biol. Chem. **236**, 536 (1961).
PRIMACK, M.P., BUCHANAN, J.L., TAPLEY, D.F.: Early stimulation of mitochondrial protein synthesis in livers from triiodothyronine injected mice. Endocrinology **87**, 1355 (1970).
RAWSON, R.W.: Physiologic effects of thyroxine in man. Proc. Mayo Clin. **39**, 637 (1964).
RICH, C., BIERMAN, E.L., SCHWARTZ, I.L.: Plasma nonesterified fatty acids in hyperthyroid states. J. clin. Invest. **38**, 275 (1959).
SCHADLOW, A.R., SURKS, M.I., SCHWARTZ, H.L., OPPENHEIMER, J.H.: Specific triiodothyronine binding sites in the anterior pituitary of the rat. Science **176**, 1252 (1972).
SNYDER, L.M., REDDY, W.J.: Mechanism of action of thyroid hormones on erythrocyte 2,3-diphosphoglyceric acid synthesis. J. clin. Invest. **49**, 1993 (1970).
SOKOLOFF, L., FRANCIS, C.M., CAMPBELL, P.L.: Thyroxine stimulation of amino acid incorporation into protein independent of any action on messenger RNA synthesis. Proc. nat. Acad. Sci. (Wash.) **52**, 728 (1964).
— KAUFMAN, S.: Thyroxine stimulation of amino acid incorporation into protein. J. biol. Chem. **236**, 795 (1961).
— — GELBOIN, H.V.: Thyroxine stimulation of soluble ribonuclei acid bound amino acid transfer to microsomal protein. Biochim. biophys. Acta (Amst.) **52**, 410 (1961).
— ROBERTS, P.A., JANUSKA, M.M., KLINE, J.E.: Mechanism of stimulation of protein synthesis by thyroid hormones *in vivo*. Proc. nat. Acad. Sci. (Wash.) **60**, 652 (1968).
STERLING, K., BRENNER, M.A., SALDANHA, V.F., MILCH, P.O.: Cytosol binding protein of thyroxine in human and rat kidney. Proc. 48th Ann. Meeting Am. Thyroid Assoc. 1972, p. 43.
STOCKER, W.W., SAMAHA, F.J., GROOT, L.J. DE: Coupled oxidative phosphorylation in muscle of thyrotoxic patients. Amer. J. med. **44**, 900 (1968).
TAPLEY, D.F.: Mode and site of action of thyroxine. Proc. Mayo Clin. **39**, 626 (1964).
— KIMBERG, D.V., BUCHANAN, J.L.: The mitochondrion. New Engl. J. Med. **276**, 1182 (1967).
TATA, J.R.: Is there a relationship between the metabolism of thyroid hormones and their action? Mem. Soc. Endocrinol. **11**, 90 (1961).
— The action of thyroid hormones. J. clin. Path. **20**, 323 (1967), Suppl.
— Thyroid hormones and the formation and distribution of ribosomes. Acta endocr. (Kbh.), Suppl. **124**, to Vol. 56 (1967) p. 141.
— Ribosomes and thyroid hormones. In: Wirkungsmechanismen der Hormone. Berlin-Heidelberg-New York: Springer 1967.
— Hormonal regulation of growth and protein synthesis. Nature (Lond.) **219**, 331 (1968).
— WIDNELL, C.C.: Ribonucleic acid synthesis during the early action of thyroid hormones. Biochem. J. **98**, 604 (1966).
VOLFIN, P., KAPLAY, S.S., SANADI, D.R.: Early effects of thyroxine *in vivo* on rapidly labeled mitochondrial protein fractions and respiratory control. J. biol. Chem. **244**, 5631 (1969).
WALLACH, S., BELLAVIA, J.V., GAMPONIA, P.J., BRISTRIM, P.: Thyroxine-induced stimulation of hepatic cell transport of calcium and magnesium. J. clin. Invest. **51**, 1572 (1972).
WANG, E.: Creatine metabolism and endocrine regulation. Acta med. scand., Suppl. **169**, 1 (1946).
WAYNE, E.J.: Clinical and metabolic studies in thyroid disease. Brit. med. J. **1960 I**, 1, 78.
WILDENTHAL, K.: Studies of isolated fetal mouse hearts in organ culture. Evidence for a direct effect of triiodothyronine in enhancing cardiac responsiveness to catecholamines. J. clin. Invest. **51**, 2702 (1972).
WILKINS, L., FLEISCHMANN, W.: Effects of thyroid on creatine metabolism with a discussion of the mechanism of storage and excretion of creatine bodies. J. clin. Invest. **25**, 360 (1946).
WOLFF, J.: The effect of thyroxine on isolated dehydrogenases. III. J. biol. Chem. **237**, 236 (1962).
WYSOCKI, S.J., SEGAL, W.: Influence of thyroid hormones on enzyme activities of myelinating rat central nervous tissue. Europ. J. Biochem. **28**, 183 (1972).
ZAIMIS, E., PAPADAKI, L., ASH, A.S.F., LARBI, E., KAKARI, S., MATTHEW, M., PARADELIS, A.: Cardiovascular effects of thyroxine. Cardiovasc. Res. **3**, 118 (1969).

Control of Thyroid Function: TRH, TSH, Autoregulation

ADAMS, D.D., KENNEDY, T.H., UTIGER, R.D.: Comparison of bioassay and immunoassay measurements of serum thyrotropin (TSH) and study of TSH-levels by immunoassay of serum concentrates. J. clin. Endocr. **34**, 1074 (1972).
ANDERSON, M.S., BOWERS, C.Y., KASTIN, A.J., SCHALCH, D.S., SCHALLY, A.V., SNYDER, P.J., UTIGER, R.D., WILBER, J.F., WISE, A.J.: Synthetic thyrotropin-releasing hormone. A potent stimulator of thyrotropin secretion in man. New Engl. J. Med. **285**, 1279 (1971).
AVERILL, R.L.W., PURVES, H.D., SIRETT, N.E.: Relation of the hypothalamus to anterior pituitary thyrotropin secretion. Endocrinology **69**, 735 (1961).
BAKKE, J.L., LAWRENCE, N.: Circadian periodicity in thyroid stimulating hormone titer in the rat hypophysis and serum. Metabolism **14**, 841 (1965).
— — ARNETT, F., MACFADDEN, W.: The Fractionation of exogenous and endogenous thyroid-stimulating hormone from human and rat plasma and tissues. J. clin. Endocr. **21**, 1280 (1961).
— — ROY, S.: Disappearance rate of exogenous thyroid-stimulating hormone (TSH) in man. J. clin. Endocr. **22**, 351 (1962).
BARAKAT, R.M., INGBAR, S.H.: The effect of acute iodide depletion on thyroid function in man. J. clin. Invest. **44**, 1117 (1965).
BATES, R.W., CONDLIFFE, P.G.: Studies on the chemistry and bioassay of thyrotropins from bovine pituitaries, transplantable pituitary tumors of mice and blood plasma. Recent Progr. Hormone Res. **16**, 309 (1960).

BATES, R., GARRISON, M. M., COOPER, J. A., CONDLIFFE, P. G.: Further studies on the purification of a human thyrotropin. Endocrinology **83**, 721 (1968).

BAUER, W. C., MEYER, J. S.: Origin and fate of colloid droplets of the thyroid gland. A study using iodine-125 electromicroscopic autoradiography. Lab. Invest. **14**, 1795 (1965).

BECKERS, C., MACHIELS, J., SOYEZ, C., CORNETTE, C.: Metabolic clearance rate and production rate of thyroid stimulating hormone in man. Horm. Metab. Res. **3**, 34 (1971).

BROWN-GRANT, K.: The "feed-back" hypothesis of the control of thyroid function. Ciba Coll. Endocr. **10**, 97 (1957).

— Hypothalamus and the thyroid gland. Brit. med. Bull. **16**, 165 (1960).

— The control of thyroid secretion. J. clin. Path. **20**, 327 (1967), Suppl.

BURKE, G.: The cell membrane: A common site of action of thyrotropin (TSH) and long-acting thyroid stimulator (LATS). Metabolism **18**, 720 (1969).

— Thyroid stimulators and thyroid stimulation. Acta Endocr. (Kbh.) **66**, 558 (1971).

BURGUS, R., DONN, TH. F., DESIDERIO, D. M., WARD, D. N., VALE, W., GUILLEMIN, R., FELIX, P. M., GILLESSEN, D., STUDER, R. O.: Biological activity of synthetic polypeptide derivatives related to the structure of hypothalamic TRF. Endocrinology **86**, 573 (1970).

CONDLIFFE, P. G., ROBBINS, J.: Pituitary thyroid stimulating hormone and other thyroid-stimulating substances. In: GRAY, C. H., and A. L. BLACHARACH, Hormones in blood, 2nd ed. London-New York: Academic Press 1967.

DEISS, W. P., JR., PEAKE, R. L.: The mechanism of thyroid hormone secretion. Ann. intern. Med. **69**, 881 (1968).

DUMONT, J. E.: The action of thyrotropin on thyroid metabolism. Vitam. and Horm. **29**, 287 (1971).

FALCONER, I. R., HETZEL, B. S.: Effect of emotional stress and TSH on thyroid vein hormone level in sheep with exteriorized thyroids. Endocrinology **75**, 42 (1964).

FIELD, J. B.: Studies on the mechanism of action of thyroid stimulating hormone. Metabolism **17**, 226 (1968).

FREINKEL, N.: Extrathyroidal actions of pituitary thyrotropin: effects on the carbohydrate, lipid and respiratory metabolism of rat adipose tissue. J. clin. Invest. **40**, 476 (1961).

GILLESSEN, D., FELIX, A. M., LERGIER, W., STUDER, R. O.: Synthese des Thyrotropin releasing-Hormons (TRH) (Schaf) und verwandter Peptide. Helv. chim. Acta **53**, 63 (1970).

GUILLEMIN, R.: Hypothalamic factors releasing pituitary hormones. Recent Progr. Hormone Res. **20**, 89 (1964).

HALL, R., AMOS, J., ORMSTON, B. J.: Radioimmunoassay of human serum thyrotropin. Brit. med. J. **1971 I**, 582.

HALMI, N. S., STUELKE, R. G.: Problems of thyroidal self regulation. Metabolism **5**, 646 (1956).

HERSHMAN, J. M., PITTMAN, J. A.: Utility of the radioimmunoassay of serum thyrotropin in man. Ann. intern. Med. **74**, 481 (1971).

— — Control of thyrotropin secretion in man. New Engl. J. Med. **285**, 997 (1971).

INGBAR, S. H.: Autoregulation of the thyroid. Response to iodide excess and depletion. Mayo Clin. Proc. **47**, 814 (1972).

ISHII, J., SHIZUME, K., OKINAKA, S.: Effect of stimulation of the vagus nerve on thyroid release of I^{131}-labelled hormones. Endocrinology **82**, 7 (1968).

LEAK, D.: The Thyroid and the Autonomic Nervous System. London: Heinemann 1970.

LEMARCHAND-BÉRAUD, TH., VANNOTTI, A.: Relationships between blood thyrotropin level, protein-bound iodine and free thyroxine concentration in man under normal physiological conditions. Acta endocr. (Kbh.) **60**, 315 (1969).

LIAO, T. H., PIERCE, J. G.: The primary structure of bovine thyrotropin. J. biol. Chem. **246**, 850 (1971).

LIBERTI, P., STANBURY, J. B.: The pharmacology of substances affecting the thyroid gland. Ann. Rev. Pharmacol. **11**, 113 (1971).

MAAYAN, M. L., INGBAR, S. H.: Epinephrine: effect on uptake of iodine by dispersed cells of calf thyroid gland. Science **162**, 124 (1968).

— MILLER, S. L., INGBAR, S. H.: Effects of serotonin on iodide and intermediary metabolism in isolated thyroid cells. Endocrinology **88**, 620 (1971).

MAYBERRY, W. E., GHARIB, H., BISTAD, J. M., SIZEMORE, G. W.: Radioimmunoassay for human thyrotropin. Clinical value in patients with normal and abnormal thyroid function. Ann. intern. Med. **74**, 471 (1971).

MELANDER, A., SUNDLER, F.: Interactions between catecholamines, 5-hydroxytryptamine and TSH on the secretion of thyroid hormone. Endocrinology **90**, 188 (1972).

NAIR, R. M. G., BARRETT, J. F., BOWERS, C. Y., SCHALLY, A. V.: Structure of porcine thyrotropin releasing hormone. Biochemistry **9**, 1103 (1970).

NICOLOFF, J. T.: A new method for the measurement of thyroidal iodine release in man. J. clin. Invest. **49**, 1912 (1970).

ODELL, W. D., WILBER, J. F., UTIGER, R.: Studies of thyrotropin physiology by means of radioimmunoassay. Recent Progr. Hormone Res. **23**, 47 (1967).

PATEL, Y. C., BURGER, H. G., HUDSON, B.: Radioimmunoassay of serum thyrotropin: sensitivity and specificity. J. clin. Endocr. **33**, 768 (1971).

PIERCE, J. G., BAHL, O.-P., CORNELL, J. S., SWAMINATHAN, N.: Biologically active hormones prepared by recombination of the L-chain of human chorionic gonadotropin and the hormone-specific chain of bovine thyrotropin or of bovine luteinizing hormone. J. biol. Chem. **246**, 2321 (1971).

PURVES, H. D.: On the mechanism of hypothalamic control of thyrotropin secretion. Acta endocr. (Kbh.), Suppl. **50**, 21 (1960).

RAPPAPORT, L., LETERRIER, J., NUNEZ, J.: Soluble phosphoprotein kinases from thyroid tissue. Biochimie **53**, 721 (1971).

REICHERT, L. E., JR.: On the relationship between human thyrotropin research standard A, the United States Pharmacopeia thyrotropin standard (bovine) and the international standard for thyrotrophin (bovine). J. clin. Endocr. **31**, 331 (1970).

REICHLIN, S., UTIGER, R. D.: Regulation of the pituitary-thyroid axis in man: relationship of TSH concentration to concentration of free and total thyroxine in plasma. J. clin. Endocr. **27**, 251 (1967).

SCHREIBER, V.: Thyrotrophin-releasing factor: purification, mechanism and blockage of action (a review). Neuroendocrinology **2**, 175 (1967).

SÖDERBERG, U.: Temporal characteristics of thyroid activity. Phys. Rev. **39**, 777 (1959).

STEIGER, H., STUDER, H., GREER, M. A., KOHLER, H.: *In vivo* relationship between T_4 and T_3 synthesis in the rat thyroid gland and their secretion and concentration in thyroid vein and aortic blood. Acta endocr. (Kbh.) (1969).

STUDER, H.: Die Regulation der Schilddrüsenfunktion bei Jodmangel. Bern: Huber 1966.

— GREER, M. A.: A study of the mechanism involved in the production of iodine deficiency goiter. Acta endocr. (Kbh.) **49**, 616 (1965).

STUDER, R., WERNER, I.: Thyrotropin-releasing factor (TRF). Schweiz. med. Wschr. **100**, 1086 (1970).

TAUROG, A., THIO, D. T.: TSH-induced thyroxine release from puromycin-blocked thyroid glands of intact rabbits. Endocrinology **78**, 103 (1966).

VANHAELST, L., VAN CAUTER, E., DEGAUTE, J. P., GOLSTEIN, J.: Circadian variations of serum thyrotropin levels in man. J. clin. Endocr. **35**, 479 (1972).

WEBSTER, B. R., GUANSING, A. R., PAICE, J. C.: Absence of diurnal variation of serum TSH. J. clin. Endocr. **34**, 899 (1972).

WERNER, S.C.: Thyrotropin. Springfeld: Thomas 1963.
WILBER, J.F., BAUM, D.: Elevation of plasma TSH during surgical hypothermia. J. clin. Endocr. **31**, 372 (1970).
— PORTER, J.C.: Thyrotropin and growth hormone releasing activity in hypophyseal portal blood. Endocrinology **87**, 807 (1970).
ZOR, U., KANEKO, T., LOWE, I.P., BLOOM, G., FIELD, J.B.: Effect of thyroid stimulating hormone and prostaglandins on thyroidal adenyl cyclase activation and cyclic adenine 3′,5′-monophosphate. J. biol. Chem. **244**, 5189 (1969).

Relationship of Thyroid Gland to Adrenal Medulla and Catecholamines

BRAY, G.A.: Studies on the interactions of thyroid hormone and catecholamines. J. clin. Invest. **43**, 285 (1964).
BREWSTER, W.R., JR., ISAACS, J.P., OSGOOD, P.F., KING, T.L.: The hemodynamic and metabolic interrelationships in the activity of epinephrine, norepinephrine and the thyroid hormones. Circulation **13**, 1 (1956).
CULLHED, I., PARROW, A.: Acute hemodynamic changes following betaadrenergic blockade in hyperthyroidism. Acta med. scand. **184**, 235 (1968).
DE GROOT, W.J., LEONARD, J.J.: Hyperthyroidism as a high cardiac output state. Amer. Heart J. **79**, 265 (1970).
FALCONER, I.R.: Effects of fear and adrenaline on blood flow from the thyroid vein in sheep with exteriorized thyroids. J. Physiol. (Lond.) **177**, 215 (1965).
— The effect of adrenaline and noradrenaline on hormone secretion and blood flow from the thyroid veins in sheep with exteriorized thyroids. J. Physiol. (Lond.) **188**, 425 (1967).
GAFFNEY, T.E., BRAUNWALD, E., KAHLER, R.L.: Effects of guanethidine on triiodothyronine-induced hyperthyroidism in man. New Engl. J. Med. **265**, 16 (1961).
GALTON, V.A.: Thyroid hormone-catecholamine relationship. Endocrinology **77**, 278 (1965).
GROSSMAN, W., ROBIN, N.J., JOHNSON, L.W., BROOKS, H.L., SELENKOW, H.A., DEXTER, L.: The enhanced myocardial contractility of thyrotoxicosis. Ann. intern. Med. **74**, 869 (1971).
HARRISON, T.S.: Adrenal medullary and thyroid relationships. Phys. Rev. **44**, 161 (1964).
HOWITT, G., ROCOLANDS, D.J.: The heart in hyperthyroidism. Amer. Heart J. **73**, 282 (1967).
LEAK, D.: Adrenergic blockade and thyrotoxicosis. Acta endocr. (Kbh.) **43**, 131 (1963).
— The thyroid and the autonomic nervous system. London: Heinemann 1970.
LEE, W.Y., BRONSKY, D., WALDSTEIN, S.S.: Studies of thyroid and sympathetic nervous system interrelationships. II. Effects of guandethidine on manifestations of hyperthyroidism. J. clin. Endocr. **22**, 879 (1962).
MOWBRAY, J.F., PEART, W.S.: Effects of noradrenaline and adrenaline on the thyroid. J. Physiol. (Lond.) **151**, 261 (1960).
PARSONS, V., RAMSAY, I.: Thyroid and adrenal relationships. Postgrad. med. J. **44**, 377 (1968).
PIETRAS, R.J., REAL, M.A., POTICHA, G.S., BRONSKY, D., WALDSTEIN, S.S.: Cardiovascular response in hyperthyroidism. The influence of adrenergic receptor blockade. Arch. intern. Med. **129**, 426 (1972).
WALDSTEIN, S.S.: Thyroid-catecholamine interrelations. Ann. Rev. med. **17**, 123 (1966).
WILSON, W.R., THEILEN, E.O., FLETCHER, F.W.: Pharmacodynamic effects of β-adrenergic receptor blockade in patients with hyperthyroidism. J. clin. Invest. **43**, 1697 (1964).
— — HEGE, J.H., VALENCA, M.R.: Effects of β-adrenergic receptor blockade in normal subjects before, during and after triiodothyronine induced hypermetabolism. J. clin. Invest. **45**, 1159 (1966).
ZAIMIS, E., PAPADAKI, L., ASH, A.S.F., LARBI, E., KAKARI, S., MATTHEW, M., PARADELIS, A.: Cardiovascular effects of thyroxine. Cardiovasc. Res. **3**, 118 (1969).

Relationship of the Thyroid Gland to Adrenal Cortex

BROWN-GRANT, K.: Inhibition of the release of thyroidal radioiodine in the rat by cortisone. Endocrinology **56**, 607 (1955).
FELBER, J.P., REDDY, W.J., SELENKOW, H.A., THORN, C.W.: Adrenocortical response to the 48-hour ACTH test in myxedema and hyperthyroidism. J. clin. Endocr. **19**, 895 (1959).
FREDRICKSON, D.S., FORSHAM, P.H., THORN, G.W.: The effect of massive cortisone therapy on measurements of thyroid function. J. clin. Endocr. **12**, 541 (1952).
HELLMAN, L., BRADLOW, H.L., ZUMOFF, B., GALLAGHER, T.F.: Influence of thyroid hormone on hydrocortisone production and metabolism. J. clin. Endocr. **21**, 1231 (1961).
HERNBERG, C.A., LAMBERG, B.A.: Adrenal function and thyrotoxicosis. Acta endocr. (Kbh.) **18**, 4 (1955).
PETERSON, R.E.: The influence of the thyroid on adrenal cortical function. J. clin. Invest. **37**, 736 (1958).
TOMKINS, G., MCGUIRE, J.M., JR.: The effect of thyroid hormones on adrenal steroid metabolism. Ann. N.Y. Acad. Sci. **86**, 600 (1960).
YATES, F.E., URQUART, J., HERBST, A.L.: Effects of thyroid hormones on ring A reduction of cortisone by liver. Amer. J. Physiol. **195**, 373 (1958).
ZINGG, W., PERRY, W.F.: The influence of adrenal and gonadal steroids on the uptake of iodine by the thyroid gland. J. clin. Endocr. **13**, 712 (1953).

Relationship of the Thyroid Gland to Gonads Placenta and Pregnancy

DOWLING, J.T., FREINKEL, N., INGBAR, S.H.: Effect of diethylstilboestrol on the binding of thyroxine in serum. J. clin. Endocr. **16**, 1491 (1956).
INGBAR, S.H., FREINKEL, N.: Iodine metabolism in hydatiform mole and choriocarinoma. J. clin. Endocr. **20**, 1 (1960).
ENGBRING, N.H., ENGSTROM, W.W.: Effects of estrogen and testosterone on circulating thyroid hormone. J. clin. Endocr. **19**, 783 (1959).
FEDERMAN, D.D., TOBBINS, J., RALL, J.E.: Effects of methyltestosterone on thyroid function, thyroxine metabolism and thyroxine-binding protein. J. clin. Invest. **37**, 1024 (1958).
GREENBERG, A.H., CERNICHOW, P., REBA, R.C., TYSON, J., BLIZZARD, R.M.: Observations on the maturation of thyroid function in early fetal life. J. clin. Invest. **49**, 1790 (1970).
HELLMAN, L., BRADLOW, H.L., ZUMOFF, B., FUKUSHIMA, D.K., GALLAGHER, T.F.: Thyroid-androgen interrelations and the hypocholestermic effect of androsterone. J. clin. Endocr. **19**, 939 (1959).
HOLLANDER, CH.S., GARCIA, A.M., STURGIS, S.H., SELENKOW, H.A.: Effect of an ovulatory suppressant on the serum protein-bound iodine and the red-cell uptake of radioactive tri-iodothyronine. New Engl. J. Med. **269**, 501 (1963).
JENSEN, S.E.: Studies of the effect of diethylstiltroestrol on the plasma lipids and thyroid function. Acta med. scand., Suppl. 346 to Bd. 164 (1959).
KEITEL, H.G., SHERER, M.G.: Marked depression of the plasma protein bound iodine concentration in the absence of clinical hypothyroidism during testosterone medication. J. clin. Endcr. **17**, 854 (1957).

MYANT, N.B.: Passage of thyroxine and triiodothyronine from mother to fetus in pregnant women. Clin. Sci. **17**, 75 (1958).
RAITI, S., HOLZMAN, G.B., SCOTT, R.L., BLIZZARD, R.M.: Evidence for the placental transfer of triiodothyronine in human beingn New Engl. J. Med. **277**, 456 (1967).
ROBIN, N.I., SELENKOW, H.A., FANG, U.S., REFETOFF, S., PIASECKI, G., RAUSCHECKER, H., JACKSON, B.T.: Bidirectional thyroxine exchange in pregnant sheep. Hormones **3**, 235 (1972).
ROSENBERG, I.N., AHN, C.S., MITCHELL, M.L.: Effects of anabolic steroids upon circulating thyroid hormones. J. clin. Endocr. **22**, 612 (1962).
STEIN, R.B., NICOLOFF, J.T.: Thyroid function in pregnancy. Postgrad. Med. **53**, 72 (1973).

Changes of Thyroid Function in Disease of Other Organs

CRUCHAUD, S., MAHAIM, C., SCAZZIGA, B., VANNOTTI, A.: Fonction thyroidienne et néphrose lipoïdique. Schweiz. med. Wschr. **84**, 478 (1954).
FANKHAUSER, S., HUBER, D., STUDER, H.: Zur Beurteilung der Schilddrüsenfunktion bei Patienten mit Niereninsuffizienz. Schweiz. med. Wschr. **98**, 698 (1968).
HERRMANN, J., BAHLMANN, J., KRÜSKEMPER, H.L.: Das Verhalten des Thyroxins sowie des Thyroxin-bindenden Globulins in Serum und Urin von Patienten mit renalen Thyroxinverlusten infolge nephrotischen Syndroms unterschiedlicher Genese. Acta endocr. (Kbh.) **69**, 13 (1972).
HOLLANDER, D., MEEK, J.C., MANNING, R.T.: Free thyroxine in serum of patients with cirrhosis of the liver. New Engl. J. Med. **276**, 900 (1967).
IFF, H.W., STUDER, H.: Passage of pituitary hormones through the nephrotic rat kidney and its possible biological significance. Experientia (Basel) **25**, 1151 (1969).
INADA, M., STERLING, K.: Thyroxine turnover and transport in Laënnec's cirrhosis of the liver. J. clin. Invest. **46**, 1275 (1967).
MCCONNON, J., ROW, V.V., VOLPÉ, R.: The influence of liver damage in man on the distribution and disposal rates of thyroxine and triiodothyronine. J. clin. Endocr. **34**, 144 (1972).
ODDIE, T.H., FLANIGAN, W.J., FISHER, D.A.: Iodine and thyroxine metabolism in anephric patients receiving chronic peritoneal dialysis. J. clin. Endocr. **31**, 277 (1970).
RASMUSSEN, H.: Thyroxine metabolism in the nephrotic syndrome. J. clin. Invest. **35**, 792 (1956).
VANNOTTI, A., BÉRAUD, T.: Functional relationship between the liver, the thyroxine-binding protein of serum and the thyroid. J. clin. Endocr. **19**, 466 (1959).

Hypothyroidism in the Adult

Classification, Etiology and Incidence of Hypothyroidism

ANDERSON, J.R., BUCHANAN, W.W., GOUDIE, R.B.: Autoimmunity, clinical and experimental. Springfield: Thomas 1967.
BASTÉNIE, P.A., ERMANS, A.M.: Thyroiditis and thyroid function. Oxford: Pergamon Press 1972.
— VANHAELST, L., BONNYNS, M., NÈVE, P., STAQUET, M.: Preclinical hypothyroidism: a risk factor for coronary heart disease. Lancet **1971 I**, 203.
BEGG, T.B., HALL, R.: Iodide goiter and hypothyroidism. Quart. J. Med. **32**, 251 (1963).
BERENS, S.C., WOLFF, J., MURPHY, D.L.: Lithium concentration by the thyroid. Endocrinology **87**, 1085 (1970).
BRAVERMAN, L.E., INGBAR, S.H., VAGENAKIS, A.G., ADAMS, L., MALOOF, F.: Enhanced susceptibility to iodide myxedema in patients with Hashimoto's disease. J. clin. Endocr. **32**, 515 (1971).
— WOEBER, K.A., INGBAR, S.H.: Induction of myxedema by iodide in patients euthyroid after treatment of toxic goiter. New. Engl. J. Med. **281**, 816 (1969).
BURROW, G.N., BURKE, W.R., HIMMELHOCH, J.M., SPENCER, R.P., HERSHMAN, J.M.: Effect of lithium on thyroid function. J. clin. Endocr. **32**, 647 (1971).
CANDY, J.: Severe hypothyroidism—an early complication of lithium therapy. Brit. med. J. **1972 III**, 277.
DONIACH, D., ROITT, I.M., TAYLOR, K.B.: Autoimmune phenomena in pernicious anaemia. Serological overlap with thyroiditis, thyrotoxicosis and systemic lupus erythematodes. Brit. med. J. **1963 I**, 1374.
GLATSTEIN, E., MCHARDY-YOUNG, S., BRAST, N., ELTRINGHAM, J.E., KRISS, J.P.: Alterations in serum thyrotropin (TSH) and thyroid function following radiotherapy in patients with malignant lymphoma. J. clin. Endocr. **32**, 833 (1971).
GLENNON, J.A., GORDON, E.S., SAWIN, C.T.: Hypothyroidism after low dose ^{131}I treatment of hyperthyroidism. Ann. intern. Med. **76**, 721 (1972).
HARGREAVES, A.W., GARNER, A.: Significance of lymphocytic infiltration of thyroid gland in thyrotoxicosis. Brit. J. Surg. **55**, 543 (1968).
HARRISON, M.T., ALEXANDER, W.D., HARDEN, R.M.: Thyroid function and iodine metabolism in iodine induced hypothyroidism. Lancet **1963 I**, 1238.
HERSHMAN, J.M., KONERDING, K.: Effects of sulfonylurea drugs on thyroid and serum protein-binding of thyroxine in the rat. Endocrinology **83**, 74 (1968).
HUNTON, R.B., WELLS, M.V., SKIPPER, E.W.: Hypothyroidism in diabetics treated with sulphonylurea. Lancet **1965 II**, 449.
IVY, H.K.: Permanent myxedema: an unusual complication of granulomatous thyroiditis. J. clin. Endocr. **21**, 1384 (1961).
KOCHER, TH.: Über Kropfextirpation und ihre Folgen. Langenbecks Arch. klin. Chir. **29**, 254 (1883).
LAZARUS, J.H., BENNIE, E.H.: Effect of lithium on thyroid function in man. Acta endocr. (Kbh.) **70**, 266 (1972).
MARKSON, J.L., FLATMAN, G.E.: Myxoedema after deep x-ray therapy to the neck. Brit. med. J. **1965 I**, 1228.
— MOORE, J.M.: Thyroid auto-antibodies in pernicious anaemia. Brit. med. J. **1962 II**, 1352.
MEANS, J.H., DE GROOT, L.J., STANBURY, J.B.: The thyroid and its diseases. New York: McGraw-Hill 1963.
MICHIE, W., PEGG, C.A.S., BEWSHER, P.D.: Prediction of hypothyroidism after partial thyroidectomy for thyrotoxicosis. Brit. med. J. **1972 I**, 13.
MURRAY, I.P.C., STEWART, R.D.H.: Iodide goiter. Lancet **1967 I**, 922.
PARIS, J., MCCONAHEY, W.M., QWEN, C.A., JR., WOOLNER, L.B., BATUR, R.C.: Iodide goiter. J. clin. Endocr. **20**, 57 (1960).
PASTERNAK, D.P., SOKOLOW, E.L., INGBAR, S.H.: Synergistic action of phenazone and iodide on thyroid hormone biosynthesis in the rat. Endocrinology **84**, 769 (1969).
RALLISON, M.L., KUMAGAI, L.F., TYLER, F.H.: Goitrous hypothyroidism induced by amino-glutethimide, anticonvulsant drug. J. clin. Endocr. **27**, 265 (1967).
SCHOU, M., AMDISEN, A., JENSEN, S.E., OLSON, T.: Occurrence of goiter during lithium treatment. Brit. med. J. **1968 III**, 710.
SUZUKI, H.T., HIGUCKI, S.K., OKTAKI, S., HOCIUCHI, Y.: "Endemic coast goiter" in Hokkaido Japan, Acta endocr. (Kbh.) **50**, 161 (1965).
WERNER, S.C.: Classification of thyroid disease. J. clin. Endocr. **29**, 860 (1969).
WILLIAMS, J.A., BERENS, S.C., WOLFF, J.: Thyroid secretion *in vitro*: inhibition of TSH and dibutyryl cyclic AMP

stimulated ^{131}I release by lithium. Endocrinology **88**, 1385 (1971).
VAGENAKIS, A.C., DOWNS, P., BRAVERMAN, L.E., BURGER, A., INGBAR, S.H.: Control of thyroid hormone secretion in normal subjects receiving iodides. J. clin. Invest. **52**, 528 (1973).
WOLFF, J.: Iodide goiter and the pharmacologic effects of excess iodide. Amer. J. Med. **47**, 101 (1969).

Pathologic Anatomy of Various Organs im Hypothyroidism

ANDERSEN, H., ASBOE-HANSEN, G., QUAADE, F.: Histopathologic examination of the skin in the diagnosis of myxedema in children. J. clin. Endocr. **15**, 459 (1955).
DOERR, W., HOLLDECK, K.: Ueber das Myxoedemherz. Virchows Arch. path. Anat. **315**, 653 (1948).
GABRILOVE, J.L., LUDWIG, A.W.: The histogenesis of myxedema. J. clin. Endocr. **17**, 925 (1957).
EZRIN, C., SWANSON, H.E., HUMPHREY, J.G., DAWSON, J.W., HILL, F.M.: The cells of the human adenohypophysis in thyroid disorders. J. clin. Endocr. **19**, 958 (1959).
MÖSLI, P., HEDINGER, CHR.: Noduläre Hyperplasie und Adenome des Hypophysenvorderlappens bei Hypothyreose. Acta endocr. (Kbh.) **58**, 507 (1968).
PEARSE, A.G.E.: Cytochemical localisation of the protein hormones of the anterior hypophysis. Ciba Found. Coll. Endocr. **4**, 1 (1952).
— Histochemistry, 2nd ed. London: Churchill 1960.
RUSSFIELD, A.B.: Histology of the human hypophysis in thyroid disease—Hypothyroidism, hyperthyroidism and cancer. J. clin. Endocr. **15**, 1393 (1955).

Clinical Picture, Differential Diagnosis, Course and Treatment of Adult Hypothyroidism

AIKAWA, J.K.: The nature of myxedema: Alterations in the serum electrolyte concentrations and radiosodium space and in the exchangeable sodium and potassium contents. Ann. intern. Med. **44**, 30 (1956).
ARDEMAN, S., CHAMARIN, I., KRAFCHICK, B., SINGER, W.: Addisonian pernicious anaemia and intrinsic factor antibodies in thyroid disorders. Quart. J. Med. N.S. **35**, 421 (1966).
ASPER, S.P., WISWELL, J.G.: Physiology and treatment of myxedema. Amer. J. Med. **20**, 732 (1956).
AXELROD, A.R., BERMAN, L.: The bone marrow in hyperthyroidism and hypothyroidism. Blood **6**, 436 (1951).
BALZE, F.A. DE LA, ARRILLAGA, F., MANCINI, R.E., JANCHES, M., DAVIDSON, O.W., GURTMAN, A.I.: Male hypogonadism in hypothyroidism: A study of six cases. J. clin. Endocr. **22**, 121 (1962).
BASTÉNIE, P.A., NÈVE, P., BONNYNS, M., VANHAELST, L., CHAILLY, M.: Clinical and pathological significance of asymptomatic atrophic thyroiditis. Lancet **1967 I**, 915.
— VANHAELST, L., BONNYNS, M., NÈVE, P., STAQUET, M.: Preclinical hypothyroidism: A risk factor for coronary heart disease. Lancet **1971 I**, 203.
BERNSTEIN, R.S., ROBBINS, J.: Intermittent therapy with L-thyroxine. New Engl. J. Med. **281**, 1444 (1969).
BLACK, M.M., BORROMS, E., SHUSTER, S.: Skin collagen and thickness in hyperthyroidism and myxedema. Clin. Endocr. **1**, 253 (1972).
BLAND, J.H., TRYMOYER, J.W.: Rheumatic syndromes of myxedema. New Engl. J. Med. **282**, 1171 (1970).
BILLEWICZ, W.Z., CHAPMAN, R.S., CROOKS, J., DAY, M.E., GOSSAGE, J., WAYNE, E., YOUNG, J.A.: Statistical methods applied to the diagnosis of thyroid disease. Quart. J. Med., N.S. **38**, 255 (1969).
BLEULER, M.: Endokrinologische Psychiatrie. In: Psychiatrie der Gegenwart, GRUHLE, JUNG, MAYER-GROSS u. MÜLLER, (eds.). Berlin-Göttingen-Heidelberg: Springer 1964.
BLOOMER, H.A., KYLE, L.H.: Myxedema. Arch. intern. Med. **104**, 234 (1959).
BRAUNMAN, H., CORVILAIN, J.: Growth hormone response to hypoglycemia in myxedema. J. clin. Endocr. **28**, 301 (1968).
BRONSKY, D., MELTZER, J.L., WALDSTEIN, S.S.: Idiopathic juvenile myxedema and myasthenia gravis. Amer. J. Med. **43**, 956 (1967).
BURGESS, A.M.: Myxedema—controlled by thyroid extract for fifty-two years; report of a case. Ann. intern. Med. **25**, 146 (1946).
CASSANO, C., FABBRINI, A., ANDRES, G.A., CINOTTI, G.A., MARTINO, C. DE, MINIO, F.: Functional, light and electron microscopic studies of the kidney in myxoedema. Europ. Rev. Endocr. **1**, 1 (1964).
CASSIDY, C.E., EDDY, R.L.: Hypothyroidism in patients with goiter. Metabolism **19**, 751 (1970).
CHEW, C.U., PAIN, R.W.: A comparison of thyroid function tests. Med. J. Aust. **1962 I**, 435.
DERUBERTIS, F.R., MICHELIS, M.F., BLOOM, M.E., MINTZ, D.H., FIELD, J.B., DAVIS, B.B.: Impaired water excretion in myxedema. Amer. J. Med. **51**, 41 (1971).
DISCALA, V.A., KINNEY, M.J.: Effects of myxedema on the renal diluting and concentrating mechanism. Amer. J. Med. **50**, 325 (1971).
EDWARDS, C.R.W., FORSYTH, I.A., BESSER, G.M.: Amenorrhoea, galactorrhoea, and primary hypothyroidism with high circulating levels of prolactin. Brit. med. J. **1971 III**, 462.
EVERED, D., YOUNG, E.T., ORMSTON, B.J., MENZIES, R., SMITH, P.A., HALL, R.: Treatment of hypothyroidism: a reapraisal of thyroxine therapy. Brit. med. J. **1973 III**, 131.
FITZGERALD, L.T., WILLIAMS, C.M.: A computer program for the diagnosis of thyroid disease. Amer. J. Roentgenol. **97**, 901 (1966).
FORD, R.V., OWENS, J.C., CURD, G.W., JR., MOYER, J.H., SPURR, C.L.: Kidney function in various thyroid states. J. clin. Endocr. **21**, 549 (1961).
FOWLER, P.B.S., SWALE, J.: Premyxoedema and coronary artery disease. Lancet **1967 I**, 1077.
— — ANDREWS, H.: Hypercholesterolemia in borderline hypothyroidism. Lancet. **1970 II**, 488.
FRAIGU, P., THOUIN, A., BAZIN, J.-P., TUBIANA, M.: Comparaison de la valeur diagnostique de différents tests fonctionnels thyroïdiens. Ann. Endocr. (Paris) **33**, 5 (1972).
FUHR, J.E., MEDICI, P.: An *in vitro* effect of thyroid hormone upon bone marrow synthesis of hemoglobin. FEBS Letter **11**, 20 (1970).
GORDON, A., GROVER, N.B., BARZILAI, D., EHRENFELD, E.N., LEWITUS, Z., GROSS, J.: Computer study of thyroid diseases. II Results. Israel J. med. Sci. **6**, 333 (1970).
GRAETTINGER, J.S., MUENSTER, J.J., CHECCHIA, C.S., GRISSOM, R.L., CAMPBELL, J.A.: A correlation of clinical and hemodynamic studies in patients with hypothyroidism. J. clin. Invest. **37**, 502 (1958).
GRAIG, F.A., SMITH, J.C.: Serum creatine phosphokinase activity in altered thyroid states. J. clin. Endocr. **25**, 723 (1965).
HEINONEN, O.P., GORDIN, A., AHO, K., PUNSAR, S., PYORALA, K.: Symptomless autoimmune thyroiditis in coronary heart disease. Lancet **1972 I**, 785.
HAYS, M.T.: Absorption of oral thyroxine in man. J. clin. Endocr. **28**, 749 (1968).
IRVINE, W.J., DAVIES, S.H., DELAMORE, K.W., WILLIAMS, A.W.: Immunological relationship between anaemia and thyroid disease. Brit. med. J. **1962 II**, 454.
IVY, H.K.: Myxedema precoma, complications and therapy. Proc. Mayo Clin. **40**, 403 (1965).
JELLINEK, E.H.: Fits, faints, coma and dementia in myxedema. Lancet **1962 II**, 1010.
— KELLY, R.E.: Cerebellar syndrome in myxoedema. Lancet **1960 II**, 225.

KEKKI, M.: Serum protein turnover in experimental hypo- and hyperthyroidism. Acta endocr. (Kbh.), Suppl. **91**, 1 (1964).
KIELY, J.M., PURNELL, D.C., OWEN, CH. A.: Erythrokinetics in myxedema. Ann. intern. Med. **67**, 533 (1967).
KIRKEBY, K.: Fatty acid composition of serum lipids in hyper- and hypothyroidism. Acta endocr. (Kbh.) **71**, 62 (1972).
KLION, F.M., SEGAL, R., SCHAFFNER, F.: The effect of altered thyroid function on the ultrastructure of the human liver. Amer. J. Med. **50**, 317 (1971).
KOCEN, R.S., ATKINSON, M.: Ascites in hypothyroidism. Lancet **1963 I**, 527.
KÖNIG, M.P., SCHMIDHAUSER, M.: Neurologische Störungen als Leitsymptom einer langdauernden Hypothyreose mit Tod in Myxodemcoma. Schweiz. med. Wschr. **93**, 1083 (1963).
LAMBERG, B.A., GRÄSBECK, R.: The serum protein pattern in disorders of thyroid function. Acta endocr. (Kbh.) **19**, 91 (1955).
LAVE, C.E., BIRD, E.D., THOMAS, W.C.: Hypercalcemia in myxedema. J. clin. Endocr. **22**, 261 (1962).
LEE, W.Y., MORIMOTO, P.K., BRONSKY, D., WALDSTEIN, S.S.: Studies of thyroid and sympathetic system interrelationships. I. The blepharoptosis of myxedema. J. clin. Endocr. **21**, 1402 (1961).
LEEPER, R.D., BENUA, R.S., BRENER, J.L., RAWSON, R.W.: Hyperuricemia in myxedema. J. clin. Endocr. **20**, 1457 (1960).
LEITHOLD, S.L., DAVID, D., BEST, W.R.: Hypothyroidism with anemia demonstrating abnormal vitamin B12 absorption. Amer. J. Med. **24**, 535 (1958).
LILLINGTON, G.A., GASTINEAU, C.F., UNDERDAHL, L.O.: The sedimentation rate in primary myxedema. Proc. Mayo Clin. **34**, 605 (1959).
MCBIEN, D.J., HINDLE, W.: Myxedema and heart failure. Lancet **1963 I**, 1066.
MCCONNON, J., ROW, V.V., VOLPÉ, R.: Simultaneous comparative studies of thyroxine and trioodothyronine production rates in health and disease. In: Further Adv. Thyroid Research (K. FELLINGER, R. HÖFER, eds.), p. 335. Wiener Medizinische Akademie 1971.
MEANS, M.A., DOBSON, R.L.: Cytological changes in the sweat gland in hypothyroidism. J. Amer. med. Ass. **186**, 113 (1963).
NIKKILÄ, E.A., KEKKI, M.: Plasma triclyceride metabolism in thyroid disease. J. clin. Invest. **51**, 2103 (1972).
ODDIE, T.H., FLANIGAN, W.J., FISHER, D.A.: Iodine and thyroxine metabolism in anephric patients receiving chronic peritoneal dialysis. J. clin. Endocr. **31**, 277 (1970).
O'HARA, D.D., PORTE, D., WILLIAMS, R.H.: The effect of diet and thyroxine on plasma lipids in myxedema. Metabolism **15**, 123 (1966).
PETTINGER, W.A., TALNER, L., FERRIS, TH. F.: Inappropriate secretion of antidiuretic hormone due to myxedema. New Engl. J. Med. **272**, 362 (1965).
RAVEN, H.M.: Life history of a case of myxeodema. Brit. med. J. **1924 II**, 622.
RAVEN, TH.F.: Myxoedema treated with thyroid tabloids. Brit. med. J. **1894 I**, 12.
RAWSON, R.W., RALL, J.E., PEARSON, O.H., ROBBINS, J., POPPELL, H.F., WEST, C.D.: 1-triiodothyronine versus l-thyroxine. A comparison of their metabolic effects in human myxedema. Amer. J. med. Sci. **226**, 405 (1953).
READ, D.G., HAYS, M.T., HERSHMAN, J.M.: Absorption of oral thyroxine in hypothyroid and normal men. J. clin. Endocr. **30**, 798 (1970).
RITTER, F.N.: The effects of hypothyroidism upon the ear nose and throat. Laryngoscope (St. Louis) **67**, 1427 (1967).
RIVES, K.L., FURTH, E.D., BECKER, D.V.: Limitations of the ankle jerk test. Intercomparison with other tests of thyroid function. Ann. intern. Med. **62**, 1139 (1965).
ROSS, G.T., SCHOLZ, D.A., LAMBERG, E.H., GERACI, J.E.: Severe uterine bleeding and degenerative skeletal-muscle changes in unrecognized myxedema. J. clin. Endocr. **18**, 492 (1958).
SALOMON, M.I., DISCALA, V., GRISHMAN, E., BREUER, J., CHURG, J.: Renal lesions in hypothyroidism: a study based on kidney biopsies. Metabolism **16**, 846 (1967).
SANDERS, V.: Neurologic manifestations of myxedema. New Engl. J. Med. **266**, 547 (1962).
SCHAUB, F.: Über die Herzveränderungen bei Myxoedem und einfacher Hypothyreose. Cardiologia (Basel) **30**, 185 (1957).
SCHWARZ, K.: Therapie der Hypothyreose. Internist (Berl.) **4**, 312 (1963).
SELENKOW, H.A., WOOL, M.S.: A new synthetic thyroid hormone combination for clinical therapy. Ann. intern. Med. **67**, 90 (1967).
SMITH, R.N., TAYLOR, S.A., MASSEY, J.C.: Controlled clinical trial of combined triiodothyronine and thyroxine in the treatment of hypothyroidism. Brit. med. J. **1970 IV**, 145.
SNYDER, L.M., REDDY, W.J.: Mechanism of action of thyroid hormones on erythrocyte 2,3-diphosphoglyceric acid synthesis. J. clin. Invest. **49**, 1993 (1970).
STANBURY, J.B.: Cretinism and the fetal-maternal relationship. In: Human development and the thyroid gland. Relation to endemic cretinism (J.B. STANBURY and R.L. KROC, eds.), p. 487. New York: Plenum Press 1972.
STARR, P.: Sodium l-thyroxine. J. Amer. med. Ass. **155**, 732 (1954).
STEINBERG, A.D.: Myxedema and coronary artery disease: a comparative autopsy study. Ann. intern. Med. **68**, 338 (1968).
SURKS, M.I., SCHADLOW, A.R., OPPENHEIMER, J.H.: A new radioimmunoassay for plasma L-triiodothyronine. Measurements in thyroid disease and in patients maintained on hormonal replacement. J. clin. Invest. **51**, 3104 (1972).
TUDHOPE, G.R.: The thyroid and the blood. London: Heinemann 1969.
— WILSON, G.M.: Anemia in hypothyroidism. Quart. J. Med. **29**, 513 (1960).
— — Deficiency of vitamin B 12 in hypothyroidism. Lancet **1962 I**, 703.
TULLOCH, B.R., LEWIS, B., FRASER, T.R.: Triglyceride metabolism in thyroid disease. Lancet **1973 I**, 391.
VALLOTTON, M.B., PRETELL, J.Y., FORBES, A.P.: Distinction between idiopathic primary myxedema and secondary pituitary hypothyroidism by the presence of circulating thyroid antibodies. J. clin. Endocr. **27**, 1 (1967).
VANHAELST, L., NEVE, P., CHAILLY, P., BASTENIE, P.A.: Coronary artery disease in Hypothyroidism. Lancet **1967 II**, 800.
WALDSTEIN, S.S., BRONSKY, D., SHRIFTER, H.B., OESTER, Y.T.: The electromyogram in myxedema. Arch. intern. Med. **101**, 97 (1958).
WATANAKUNAKORN, C., HODGES, R.E., EVANS, T.C.: Myxedema. Arch. intern. Med. **116**, 183 (1965).
WILLIAMS, G.A., CRISPELL, K.R., PARSON, W.: Adrenocortical function in myxedema. J. clin. Endocr. **17**, 1347 (1957).
WILSON, W.R., BEDELL, G.N.: The pulmonary abnormalities in myxedema. J. clin. Invest. **39**, 42 (1960).
WOOL, M.S., SELENKOV, H.A.: Physiologic combinations of synthetic thyroid hormones in myxedema. Clin. Pharmacol. Ther. **6**, 710 (1965).
WYSS, F.: Hypothyreose. Schweiz. med. Wschr. **97**, 1671 (1967).

Secondary Hypothyroidism

BRAUNMAN, H., CORVILAIN, J.: Growth hormone response to hypoglycenia in myxedema. J. clin. Endocr. **38**, 301 (1968).
COSTOM, B.H., GRUMBACH, M.M., KAPLAN, S.L.: Effect of thyrotropin-releasing factor on serum thyroid stimulating hormone. An approach to distinguishing hypo-

thalamic from pituitary forms of idiopathic hypopituitary dwarfism. J. clin. Invest. **50**, 2219 (1971).
FOLEY, T. P., JACOBS, L. S., HOFFMAN, W., DAUGHADAY, W. H., BLIZZARD, R. M.: Human prolactin and thyrotropin concentrations in the serums of normal and hypopituitary children before and after thyrotropin-releasing hormone. J. clin. Invest. **51**, 2143 (1972).
— OWINGS, J., HAYFORD, J. T., BLIZZARD, R. M.: Serum thyrotropin responses to synthetic thyrotropin releasing hormone in normal children and hypopituitary patients: A new test to distinguish primary releasing hormone deficiency from primary pituitary hormone deficiency. J. clin. Invest. **51**, 431 (1972).
KAPLAN, S. L., GRUMBACH, M. M., FRIESEN, H. G., COSTOM, B. H.: Thyrotropin-releasing factor (TRF) effect on secretion of human pituitary prolactin and thyrotropin in children and in idiopathic hypopituitary dwarfism: Further evidence of hypophysiotropic hormone deficiencies. J. clin. Endocr. **35**, 825 (1972).
LESSOF, M. H., LYNE, C., MAISEY, M. N., STURGE, R. A.: Effect of thyroid failure on the pituitary—adrenal axis. Lancet **1969 I**, 642.
LI, M. C., RALL, J. E., MACLEAN, J. P., LIPSETT, M. B., RAY, B. S., PEARSON, O. H.: Thyroid function following hypophysectomy in man. J. clin. Endocr. **15**, 1228 (1955).
MIYAI, K., AZUKIZAWA, M., KUMAHRA, Y.: Familial isolated thyrotropin deficiency with cretinism. New Engl. J. Med. **285**, 1043 (1971).
ODELL, W. D.: Isolated deficiencies of anterior pituitary hormones: symptoms and diagnosis. J. Amer. med. Ass. **197**, 1006 (1966).
PITTMAN, J. A., HAIGLER, E. D., HERSHMAN, J. M., PITTMAN, C. S.: Hypothalamic hypothyroidism. New Engl. J. Med. **285**, 844 (1971).
SAMPSON, M. C., ROSE, E., HERBERT, E.: Solitary ("monotropic") thyrotropin deficiency with secondary hypothyroidism. Amer. J. Med. **17**, 871 (1954).
SAWIN, C. T., MCHUGH, J. E.: Isolated lack of thyrotropin in man. J. clin. Endocr. **26**, 955 (1966).
SHENKMAN, L., MITSUMA, T., SUPHAVAI, A., HOLLANDER, C. S.: Hypothalamic hypothyroidism. J. Amer. med. Ass. **222**, 480 (1972).
SHUMAN, CH. R.: Hypothyroidism due to thyrotropin deficiency without other manifestations of hypopituitarism. J. clin. Endocr. **13**, 795 (1953).

Borderline Hypothyroid States

DELANGE, F., CAMUS, M., ERMANS, A. M.: Circulating thyroid hormones in endemic goiter. J. clin. Endocr. **34**, 891 (1972).
GHARIB, H., WAHNER, H. W., MCCONAHEY, W. M.: Serum levels of thyroid hormones in Hashimoto's thyroiditis. Mayo Clin. Proc. **47**, 175 (1972).
GOLDBERG, M.: The case for euthyroid hypometabolism. Amer. J. med. Sci. **240**, 479 (1960).
HEDLEY, A. J., HALL, R., AMOS, J., MICHIE, W., CROOKS, J.: Serum thyrotropin levels after subtotal thyroidectomy for Graves' disease. Lancet **1971 I**, 455.
HERTOGHE: De l'hypothyroïdie bénigne ou myxoedème fruste. Nouv. Iconogr. Salpêt. Juli/Aug. (1899).
JEFFERIES, W. MCK.: Occult hypothyroidism and "metabolic insufficiency". J. chron. Dis. **14**, 582 (1961).
— KELLY, L. W., JR., LEVY, R. P., COOPER, G. W., PROUTY, R. L.: The significance of low thyroid reserve. J. clin. Endocr. **16**, 1438 (1956).
KEATING, F. R.: Metabolic insufficiency. J. clin. Endocr. **18**, 531 (1958).
KURLAND, G. S., HAMOLSKY, M. W., FREEDBERG, A. S.: Studies in non-myxedematous hypometabolism. I. The clinical syndrome and the effects of triiodothyronine alone or combined with thyroxine. J. clin. Endocr. **15**, 1345 (1955).
LEVIN, M. E.: Metabolic insufficiency: A double-blind study using triiodothyronine, thyroxine and a placebo: Psychometric evaluation of the hypometabolic patient. J. clin. Endocr. **20**, 106 (1960).
POWELL, G. F., BLIZZARD, R. M.: An attempt to establish a test for thyrotropin (TSH) reserve. J. clin. Endocr. **26**, 1389 (1966).
SCHNEEBERG, N. G., KANSAC, P. C.: Commentary on a proposed TSH reserve test. J. clin. Endocr. **26**, 579 (1966).
SIKKEMA, S. H.: Triiodothyronine in the diagnosis and treatment of hypothyroidism: Failure to demonstrate the metabolic insufficiency syndrome. J. clin. Endocr. **20**, 546 (1960).
SLINGERLAND, D. W., HERSHMAN, J. M., DELL, E. S., BURROWS, B. A.: Thyrotropin and PBI in radioiodine-treated hyperthyroid patients. J. clin. Endocr. **35**, 912 (1972).
STERLING, K., BRENNER, M. A., NEWMAN, E. S., ODELL, W. D., BELLABARBA, D.: The significance of triiodothyronine (T_3) in maintainance of euthyroid status after treatment of hyperthyroidism. J. clin. Endocr. **33**, 729 (1971).
STUDER, H., WYSS, F., IFF, H. W.: A TSH-reserve Test for detection of mild secondary hypothyroidism. J. clin. Endocr. **24**, 965 (1964).
WYSS, F., STUDER, H.: Die pathophysiologische Bedeutung der eingeschränkten Leistungsreserve des Hypophysen-Schilddrüsen-Systems. Schweiz. med. Wschr. **93**, 1057 (1963).
— — Peripherer euthyreoter Hypometabolismus oder sekundäre Hypothyreose? Schweiz. med. Wschr. **93**, 1680 (1963).

Myxedema Coma

HOLVEY, D. N., GOODNER, C. J., NICOLOFF, J. T., DOWLING, J. T.: Treatment of myxedema coma with intraveinous thyroxine. Arch. intern. Med. **113**, 89 (1964).
JELLINEK, E. H.: Fits, faints, coma, and dementia in myxoedema. Lancet **1962 II**, 1010.
LEON-SOTOMAYOR, L., BOWERS, C. Y.: Myxedema coma. Springfield: Thomas 1964.
LOVEL, T. W. I.: Myxoedema coma. Lancet **1962 I**, 823.
MASSUMI, R. A., WINNACKER, J. L.: Severe depression of the respiratory center in myxedema. Amer. J. Med. **36**, 876 (1964).
NICKERSON, J. F., HILL, S. R., MCNEIL, J. H., BARKER, S. B.: Fatal myxedema with and without coma. Ann. intern. Med. **53**, 475 (1960).
NIELSON, P. E., RANLOW, P.: Myxoedema coma. Acta endocr. (Kbh.) **45**, 353 (1964).
NORDQUVIST, P., DHUNER, K. G., STERNBERG, K., ORNDAHL, G.: Myxoedema coma and CO_2-retention. Acta med. scand. **166**, 189 (1960).
ORD, W. M.: Cases of myxoedema. Trans clin. Soc. Lond. **13**, 15 (1879—1880).
PERLMUTTER, ., COHN, H.: Myxedema crisis of pituitary or thyroid origin. Amer. J. Med. **36**, 883 (1964).
RIDGWAY, E. C., MCCANNON, J. A., BENOTTI, J., MALOOF, F.: Acute metabolic responses in myxedema to large doses of intraveinous L-thyroxine. Ann. intern. Med. **77**, 549 (1972).
VERBOV, J. L.: Modern treatment of myxoedema coma associated with hypothermia. Lancet **1964 I**, 194.

Congenital and Childhood Hypothyroidism

Reviews

ANDERSEN, H. J.: Non-goitrous hypothyroidism. In: GARDNER, L. I., Endocrine and genetic diseases of childhood. Philadelphia-London: Saunders 1969.
HAYLES, A. B.: Problems of childhood Graves' disease. Mayo Clin. Proc. **47**, 850 (1972).

HUTCHISON, J.H.: Familial goitrous hypothyroidism. In: GARDNER, L.I., Endocrine and genetic diseases of childhood. Philadelphia-London: Saunders 1969.
KLEIN, E.: Inborn errors of iodine metabolism. Acta endocr. (Kbh.). Suppl. **123** (1965).
KÖNIG, M. P.: Die kongenitale Hypothyreose und der endemische Kretinismus. Berlin-Heidelberg-New York: Springer 1968.
STANBURY, J.B.: Familial goiter. In: STANBURY, J.B.; WYNGAARDEN, J.B.; FREDRICKSON, D.S., The metabolic basis of inherited disease, 3rd ed., p. 223. New York: McGraw-Hill 1972.

Specific References

ANDERSEN, H., ASBOE-HANSEN, G., QUAADE, F.: Histopathologic examination of the skin in the diagnosis of myxedema in children. J. clin. Endocr. **15**, 459 (1955).
— Studies in hypothyroidism in children. Dissertation. Copenhagen 1960. Acta paediat. (Uppsala), Suppl. 125 (1961).
— SKINHOJ, K.: A longitudinal study of mental retardation in hypothyroid children during treatment. J. Pediat. **65**, 1095 (1964).
BAMATTER, F., FRANCESCHETTI, A., KLEIN, D.: Amélioration tardive d'un cas de nanisme athyroïdien congénital avec altération intrasellaire. Helvet. paediat. Acta **2**, 154 (1947).
BELLABARBA, D., BENARD, B., VEDY, M., GATTERAU, A.: Congenital goiter with triiodothyronine as the main circulating thyroid hormone. Horm. Metab. Res. **4**, 488 (1972).
BLIZZARD, R.M., CHANDLER, R.W., LANDING, B.H., PETTIT, M.D., WEST, C.D.: Maternal autoimmunization as a probable cause of athyrotic cretinism. New Engl. J. Med. **263**, 327 (1960).
BODE. H.H., DANON, M., WEINTRAUB, B.D., MALOOF, F., CRAWFORD, J.D.: Partial target organ resistance to thyroid hormone. J. clin. Invest. **52**, 776 (1973).
BONGIOVANNI, A.M.: The use and misuse of thyroid hormone in pediatric practice. Pediatrics **33**, 585 (1964).
BURROW, G.N., SPAULDING, S.W., ALEXANDER, N.M., BOWER, B.F.: Normal peroxidase activity in Pendred's syndrome. J. clin. Endocr. **36**, 522 (1973).
CANLORBE, P., CHEFNEUX, A.: Le réflexogramme achilléen chez l'enfant. Sem. Hôp. Paris **41**, 1253 (1965).
CARSWELL, F., KERR, M.M., HUTCHISON, J.H.: Congenital goitre and hypothyroidism produced by maternal ingestion of iodides. Lancet **1970I**, 7659.
CHAN, A.M., LYNCH, M.J.G., BAILEY, J.D., EZRIN, C., FRASER, D.: Hypothyroidism in cystinosis. A clinical, epidemiologic and histologic study involving 16 patients with cystinosis. Amer. J. Med. **48**, 678 (1970).
CHANDLER, R.W., BLIZZARD, R.M., HUNG, W., KYLE, M.: Incidence of thyrotoxic factor and other antithyroid antibodies in the mothers of cretins. New Engl. J. Med. **267**, 376 (1962).
CHILDS, B., GARDNER, L.I.: Etiological factors in sporadic cretinism. An analysis of ninety cases. Ann. hum. Genet. **19**, 90 (1954).
COURVOISIER, B., DEGROOT, L.J., STANBURY, J.B., BÉRAUD, TH., KORALNIK, L.: Insuffisiances thyroïdiennes par anomalies congénitales de la synthèse hormonale. Schweiz. med. Wschr. **89**, 973 (1959).
CROSS, H.E., HOLLANDER, C.S., RIMOIN, D.L., MCKUSICK, V.A.: Familial agoitrous cretinism accompanied by muscular hypertrophy. Pediatrics **41**, 413 (1968).
DEBRÉ, R., SÉMÉLAIGNE, G.: Syndrome of diffuse muscular hypertrophy in infants causing athletic appearance. Amer. J. Dis. Child. **50**, 1351 (1935).
DE GROOT, L.J., POSTEL, S., LITVAK, J., STANBURY, J.B.: Peptide-linked iodotyrosines and iodothyronines in blood of a patient with congenital goiter. J. clin. Endocr. **18**, 158 (1958).
— STANBURY, J.B.: The syndrome of congenital goiter with butanol-insoluble serum iodine. Amer. J. Med. **27**, 586 (1959).
DONIACH, D., NILSSON, L.R., ROITT, I.M.: Autoimmune thyroiditis in children and adolescents. II. Immunological correlations and parent study. Acta paediat. (Uppsala) **54**, 260 (1965).
DORFF, G.B.: Sporadic cretinism in one of twins. Report of cases with roentgen demonstration of osseous changes that occurred *in utero*. Amer. J. Dis. Child. **48**, 1316 (1934).
EAYRS, J.T.: Thyroid hormones and brain differentiation. Ciba Found. Study Group 18. Brain-thyroid relationship, p. 60—71. London: Churchill 1964.
FISHER, D.A., HAUMOND, G.D., PICKERING, D.E.: The hypothyroid infant and child; therapy with sodium 1-thyroxine. Amer. J. Dis. Child. **90**, 6 (1955).
FISHER, W.D., VOORHESS, M.L., GARDNER, L.I.: Congenital hypothyroidism in infants following maternal I^{131} therapy. J. Pediat. **62**, 132 (1963).
FOLEY, T.P., SCHUBERT, W.K., MARNELL, R.T., MCADAMS, A.J.: Chronic lymphocytic thyroiditis and juvenile myxedema in uniovular twins. J. Pediat. **72**, 201 (1968).
FRASER, G.R., MORGANS, M.E., TROTTER, W.R.: The syndrome of sporadic goiter and congenital deafness. Quart. J. Med. **29**, 279 (1960).
FRENCH, F.S., WYK, J.J. VAN: Fetal hypothyroidism. J. Pediat. **64**, 589 (1964).
GABR, M.: The role of thyroid dysgenesis and maldescent in the etiology of sporadic cretinism. J. Pediat. **60**, 830 (1962).
GILBOA, V., BER, A., LEWITUS, Z., HASENFRATZ, J.: Goitrous myxedema due to iodide trapping defect. Arch. intern. Med. **112**, 212 (1963).
GREIG, W.R., HENDERSON, A.S., BOYLE, J.H., MCGIRR, E.M., HUTCHISON, J.A.: Thyroid dysgenesis in two pairs of monozygotic twins and in a mother and child. J. clin. Endocr. **26**, 1309 (1966).
GRIBETZ, D., TALBOT, N.B., CRAWFORD, J.D.: Goiter due to lymphocytic thyroiditis: Its occurrence in preadolescent and adolescent girls. New Engl. J. Med. **250**, 555 (1954).
HAGEN, G.A., NIEPOMNISZCE, H., HAIBACH, H., BIGAZZI, M., HATI, R., RAPOPORT, B., JIMENEZ, C., DE GROOT, L.J., FRAWLEY, T.F.: Peroxidase deficiency in familial goiter with iodide organification defect. New Engl. J. Med. **285**, 1394 (1971).
HARNACK, G.A.v.: Eineiige Zwillinge mit diskordanter Hypothyreose. Arch. Kinderheilk. **146**, 60 (1953).
— HORST, W., LENZ, W.: Das erbliche Syndrom: Innenohrschwerhörigkeit und Jodfehlverwertung im Kropf. Dtsch. med. Wschr. **86**, 2421 (1961).
— LENTZ, W., ZUCKSCHWERDT, L.: Hormontransplantation von Schilddrüsengewebe bei einem eineiigen Zwillingspaar. Dtsch. med. Wschr. **83**, 549 (1958).
— WALLIS, H.: Zur Psychopathologie der Hypothyreose im Kindesalter. Mschr. Kinderheilk. **108**, 373 (1960).
HARRIS, F., KOUTSOULIERIS, E.: Hypothyroidism due to autoimmune thyroiditis in a young child with Down's Syndrome. Arch. Dis. Child. **42**, 449 (1967).
HUBBLE, D.: Precocious menstruation in a mongoloid child with hypothyroidism. Hormonal overlap. J. clin. Endocr. **23**, 1302 (1963).
IMBACH, T.: Normalwerte der Achillessehnenreflexzeit beim Kind. Helv. paediat. Acta **24**, 463 (1969).
IWATSUBO, H., OMORI, K., OKADA, Y., FUKUCHI, M., MIYAI, K., ABE, H., KUMAHARA, Y.: Human growth hormone secretion in primary hypothyroidism before and after treatment. J. clin. Endocr. **27**, 1751 (1967).
JENKINS, M.E.: Precocious menstruation in hypothyroidism. Amer. J. Dis. Child. **109**, 252 (1965).
JEUNE, M., BERAND, C.: Ostéopétrose myxoedémateuse. Arch. franç. Pédiat. **12**, 368 (1955).

KENDLE, F. W.: Cause of precocious puberty in female cretin. Brit. med. J. **1905 I**, 246.

KENNY, F. M., ITURZAETA, N., PREEYASOMBAT, C., TAYLOR, F. H., MIGEON, C. J.: Cortisol production rate. VII. Hypothyroidism and hyperthyroidism in infants and children. J. clin. Endocr. **27**, 1616 (1967).

KOENIG, M. P., BAUMANN, TH., SCHÄRER, K., HERREN, CH.: Familiäre kongenitale Störung der Schilddrüsenhormonsynthese. Fehlerhafte Oxydation von anorganischem zu organischem Jod. Schweiz. med. Wschr. **94**, 319 (1964).

— SCHMIDHAUSER, M.: Neurologische Störungen als Leitsymptom einer langdauernden Hypothyreose mit Tod im Myxoedem-Koma. Schweiz. med. Wschr. **93**, 1183 (1963).

LAMBERG, B. A.: Congenital euthyroid goitre and partial peripheral resistance to thyroid hormones. Lancet **1973 I**, 854.

— HINTZE, G., KARLSSON, R.: Non-butanol extractable iodine in the serum of eumetabolic adult goitre patients. Acta endocr. (Kbh.) **44**, 291 (1963).

LELONG, M., JOSEPH, R., CANLORBE, P., JOB, J. C., PLAINFONE, B.: L'hypothyroïdie par anomalie congénitale de l'hormogénèse. Arch. franç. Pédiat. **13**, 341 (1956).

LISSITZKY, S., BISMUTH, J., JAQUET, P., CASTAY, M., MICHEL-BECHET, M., KOUTRAS, D. A., PHARMAKIOTIS, A. D., MOSCHOS, A., PSARRAS, A., MALAMOS, B.: Congenital goiter with impaired thyroglobulin synthesis. J. clin. Endocr. **36**, 17 (1973).

LJUNGGREN, J. G., LINDSTRÖM, H., HJERN, B.: The concentration of peroxidase in normal and adenomatous human thyroid tissue with special reference to patients with Pendred's syndrome. Acta endocr. (Kbh.) **72**, 272 (1973).

LOWREY, G. H., ASTER, R. H., CARR, E. A., RAMON, G., BEIERWALTES, W. H.: Early diagnostic criteria of congenital hypothyroidism. Amer. J. Dis. Child. **96**, 131 (1958).

MCGIRR, E. M., CLEMENT, W. E., CURRIE, A. R., KENNEDY, J. S.: Impaired dehalogenase activity as a cause of goiter with malignant changes. Scot. med. J. **4**, 232 (1959a).

— HUTCHISON, J. H., CLEMENT, W. E., KENNEDY, J. S., CURRIE, A. R.: Goitre and cretinism due to the production of an abnormal iodinated thyroid compound. Scot. med. J. **5**, 189 (1960).

MEDEIROS, G., LEVY, J. A., ULHOA CINTRA, A. B.: Congenital hypothyroidism with dishormonogenesis accompanied by muscular dystrophy. Acta endocr. Panam. **3**, 79 (1972).

MEDEIROS-NETO, G. A., Bloise, W., ULHOA-CINTRA, A. B.: Partial defect of iodide trapping mechanism in two siblings with congenital goiter and hypothyroidism. J. clin. Endocr. **35**, 370 (1972).

MILUTINOVIC, P. S., STANBURY, J. B., WICKEN, J. V., JONES, E. W.: Thyroid function in a family with the Pendred syndrome. J. clin. Endocr. **29**, 962 (1969).

MONACO, F., ROBBINS, J.: Defective thyroglobulin synthesis in an experimental rat thyroid tumor. Lack of membrane-bound sialyltransferase activity. J. biol. Chem. **248**, 2328 (1973).

MONEY, J., LEWIS, V.: Longitudinal study of intelligence quotients in treated congenital hypothyroidism. Ciba Found. Study Group 18. Brain-thyroid relationship, p. 75—88. London: Churchill 1964.

NAJJAR, S. S., NACHMAN, H. S.: Kocher-Debré-Sémélaigne syndrome: Hypothyroidism with muscular "hypertrophy". J. Pediat. **66**, 901 (1965).

NAKAJIMA, J., NIIMI, H., FUJIMORI, M.: Defective deiodination of ^{131}I-labeled L-diiodotyrosine in a family with cases of euthyroid goiter and hyperthyroidism. Endocr. jap. **18**, 205 (1971).

NIEPOMNISZCZE, H., CASTELLS, S., DEGROOT, L. J., REFETOFF, S., KIM, O. S., RAPOPORT, B., HATI, R.: Peroxidase defect in congenital goiter with complete organification block J. clin Endocr. **36**, 347 (1973).

NIEPOMNIZCE, H., DE GROOT, L. J., HAGEN, G. A.: Abnormal thyroid peroxidase causing iodide organification defect. J. clin. Endocr. **34**, 607 (1972).

OLIVER, R. A. M., BAKER, G. P.: Juvenile pernicious anemia and hypothyroidism. A family study. Brit. med. J. **1969 I**, 27.

PENDRED, V.: Deaf-mutism and goitre. Lancet **1896 II**, 532.

PRADER, A., PERABO, F.: Körperwachstum, Knochen- und Zahnentwicklung bei der endokrinen Erkrankung im Kindesalter. Helv. paediat. Acta **7**, 517 (1952).

REFETOFF, S., DE GROOT, L. J., BENARD, B., DEWIND, L. T.: Studies of a sibship with apparent hereditary resistance to the intracellular action of thyroid hormone. Metabolism **21**, 723 (1972).

— DE WIND, L. T., DE GROOT, L. J.: Familial syndrome combining deaf-mutism. stippled epiphysis, goiter and abnormally high PBI: possible target organ refractoriness to thyroid hormone. J. clin. Endocr. **27**, 279 (1967).

REINWEIN, D.: Hormonsynthese und Enzymspektrum bei Erkrankungen der menschlichen Schilddrüse. Acta endocr. (Kbh.) **47**, Suppl. 94 (1964).

— HACKENBERG, K.: Euthyreote Struma mit Jodisationsdefekt und vorwiegender Bildung von Trijodthyronin als neuer Form von Jodfehlverwertung. Schweiz. med. Wschr. **100**, 1067 (1970).

— KLEIN, E.: Eine besondere Form von Jodfehlverwertung bei sporadischem Kretinismus. Klinische und biochemische Untersuchungsergebnisse. Schweiz. med. Wschr. **93**, 1213 (1963).

ROSSIER, A., JOB, J.-C., CANLORBE, P., PISSARO, P., RIORON, J., GALLET, P., PIZZO, P.: Myxoedeme acquis de l'enfant. Arch. franç. Pédiat. **23**, 23 (1966).

ROYER, P., MÉGEVAND, A.: Les anomalies squelettiques du myxoedème congénital et leur valeur diagnostique. Arch. franç. Pédiat. **11**, 125 (1954).

SACREZ, R., JUIF, J.-G., MANDRY, J., KELLERSHON, C., RIVIÈRE, R., COMAR, D.: Le syndrôme de Pendred. I. Etudes critiques, cliniques et génétiques. Ann. Pédiat. **43**, 5 (1967).

SAXENA, K. M., CRAWFORD, J. D.: Juvenile lymphocytic thyroiditis. Pediatrics **30**, 917 (1962).

SHIMAOKA, K.: Recurrent goiter with production of an unusual iodocompound. J. clin. Endocr. **34**, 638 (1972).

SMITH, D. W., BLIZZARD, R. M., WILKINS, L.: The mental prognosis in hypothyroidism of infancy and childhood: Review of 128 cases. Pediatrics **19**, 1011 (1957).

STANBURY, J. B., CHAPMAN, E. M.: Congenital hypothyroidism with goitre: Absence of an iodide-concentrating mechanism. Lancet **1960 II**, 1162.

— HEDGE, A. N.: A study of a family of goitrous cretins. J. clin. Endocr. **10**, 1471 (1950).

— KASSENAAR, A. A. H., MEIJER, J. W. A., TERPSTRA, J.: The occurrence of mono- and diiodotyrosine in the blood of a patient with congenital goiter. J. clin. Endocr. **15**, 1216 (1955).

— RICCABONA, G., JANSSEN, M.-A.: Iodotyrosyl coupling defect in congenital hypothyroidism with goitre. Lancet **1963 I**, 917.

— ROCMANS, P., BÜHLER, U. K., OCHI, Y.: Congenital hypothyroidism with impaired thyroid response to thyrotrophin. New Engl. J. Med. **279**, 1132 (1968).

SWOBODA, W.: Typische Ossifikationsstörung der Wirbelsäule beim angeborenen Myxödem. Helv. paediat. Acta **10**, 462 (1955).

— WOLF, H. G.: Der Icterus neonatorum prolongatus beim kongenitalen Myxödem. Neue österr. Z. Kinderheilk. **1**, 149 (1955).

VAGUE, J., LISSITZKY, S., CODACCIONI, J. L., SIMONIN, R., MILLER, G., BOYER, J., AUDIBERT, G.: Infantile hypothyroidism with goitre and defect of deiodination of iodotyrosines: treatment with Lugol's solution. Lancet **1962 I**, 1070.

VALENTA, L. J., BODE, H., VICKERY, A. L., CAULFIELD, J. B., MALOOF, F.: Lack of thyroid peroxidase activity as the cause of congenital goitrous hypothyroidism. J. clin. Endocr. **36**, 830 (1973).

WERNER, S.C., BLOCK, R.J., MANDL, R.H.: Probable genetic basis for abnormal circulating iodoprotein (butanol insoluble serum iodine): Study of a family with several hypothyroid members with and without goiter. J. clin. Endocr. **20**, 205 (1960).
WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence, 3rd ed. Springfield: Thomas 1965.
WINTER, J., EBERLEIN, W.R., BONGIOVANNI, A.M.: The relationship of juvenile hypothyroidism to chronic lymphocytic thyroiditis. J. Pediat. **69**, 709 (1966).
WOLFF, J., THOMPSON, R.H., ROBBINS, J.: Congenital goitrous cretinism due to absence of iodide-concentrating ability. J. clin. Endocr. **24**, 699 (1964).
WYK, J.J. VAN, GRUMBACH, M.: Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: An example of hormonal overlap in pituitary feedback. J. Pediat. **57**, 416 (1960).

Thyroid Ectopism and Aplasia

BERNHEIM, R.M., BERGER, M., BERTRAND, J., FRANÇOES, B.: Etude génétique de l'insuffisance thyroïdienne de l'enfant. 18. Congr. Assoc. Pédiatres Langue Française, Genève, 1961. Vol. 1., p. 249. Basel-New York: Karger 1961.
DORTA, T., BÉRAUD, TH., VANNOTTI, A.: Le métabolisme de l'iode dans la thyroïde ectopique. Schweiz. med. Wschr. **90**, 150 (1960).
HERLANT, M., LINQUETTE, M., LAINE, E., FOSSATI, P., MAY, J.P., LEGEBORE, J.: Adénome hypophysaire à cellules thyréotropes avec syndrome aménorrhéegalactorrhée chez une malade porteuse d'un myxoedème congénital par ectopie thyroïdienne. Ann. Endocr. (Paris) **27**, 181 (1966).
JOSS, E., KÖNIG, M.P.: Bedeutung der Schilddrüsenektopie bei der sporadischen kongenitalen Hypothyreose. Schweiz. med. Wschr. **96**, 722 (1966).
KEELING, J.H., OCHSNER, A.: Carcinoma in thyreoglossal remnants. Cancer (Philad.) **12**, 596 (1959).
KELLERSOHN, C., MARTIN, J.: Sur la mise en évidence par I^{131} de la fréquence des thyroïdes linguales dans le myxoedème. Acta radiol. (Stockh.) **51**, 137 (1959).
KÖNIG, M.P., ESCHER, F.: Zungengrundschilddrüsen mit verschiedener klinischer Symptomatologie. Schweiz. med. Wschr. **89**, 1234 (1959).
LITTLE, G., MEADOR, C.K., CUNNINGHAM, R., PITTMAN, J.A.: "Cryptothyroidism", the major cause of sporadic "athyreotic" cretinism. J. clin. Endocr. **25**, 1529 (1965).
MCGIRR, E.M., HUTCHISON, J.H.: Dysgenesis of the thyroid gland as a cause of cretinism and juvenile myxedema. J. clin. Endocr. **15**, 668 (1955).
NEIMANN, N., KELLERSOHN, C., PIERSON, M., MARTIN, J.: Le rôle de l'ectopie thyroïdienne dans la génèse du myxoedème. Arch. franç. Pédiat. **15**, 315 (1958).
— PIERSON, M., MARTIN, J.: Hypothyroïdies par trouble du développement et lésions inflammatoires du corps thyroïde. 18e. Congr. Ass. Pédiatres Langue franç. Genève 1961, vol. 1, p. 89. Basel: Karger 1961.
RANDOLPH, J., GRUNT, J.A., VAWTER, G.F.: The medical and surgical aspects of intratracheal goiter. New Engl. J. Med. **268**, 457 (1963).
SLOAN, L.W.: Normal and anomalous development of the thyroid: In: WERNER, S.C., The thyroid, 2nd, ed. New York: Hoeber 1962.

Thyrotoxicosis

Historical Papers and Reviews

CHVOSTEK, F.: Morbus Basedow und die Hyperthyreosen. Berlin: Springer 1917.
IRVINE, W.J.: Thyrotoxicosis. Proceedings of Symposium Edinburgh 1967. Edinburgh-London: Livingstone 1967.
MAGNUS-LEVY, A.: Über den respiratorischen Gaswechsel unter dem Einfluß der Thyreoidea sowie unter verschiedenen pathologischen Zuständen. Berl. klin. Wschr. **1895**, 650.
MOEBIUS, P.J.: Die Basedow'sche Krankheit. Bd. XXII von Nothnagels Handbuch der speziellen Pathologie und Therapie. Wien: Alfred Hölder 1896.
MÜLLER, F.: Beiträge zur Kenntnis der Basedow'schen Krankheit. Arch. klin. Med. **51**, 335 (1893).
SATTLER, H.: Die Basedow'sche Krankheit. In: GRAEF-SAEMISCH, Handbuch der gesamten Augenheilkunde. Bd. IX. Leipzig 1909.
Symposium on Graves' disease. Mayo Clin. Proc. **47**, 801 (1972).

Definition, Classification, Thyroid Hormone Kinetics

HOCH, F.: Biochemistry of Hyperthyroidism and hypothyroidism. Postgrad. med. J. **44**, 347 (1968).
HOCH, F.L.: Thyrotoxicosis as a disease of mitochondria. New Engl. J. Med. **266**, 446 (1962).
INADA, M., STERLING, K.: Thyroxine transport in thyrotoxicosis and hypothyroidism. J. clin. Invest **46**, 1442 (1967).
INGBAR, S.H., FREINKEL, N.: Studies of thyroid function and the peripheral metabolism of I^{131} labelled thyroxine in patients with treated Graves' disease. J. clin. Invest **37**, 1603 (1958).
MCCONNON, J., ROW, V.V., VOLPE, R.: Simultaneous comparative studies of thyroxine and triiodothyronine distribution and disposal rates. J. Endocr. **51**, 17 (1971).
NICOLOFF, J.T., LOW, J.C., DUSSAULT, J.H., FISHER, D.A.: Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man. J. clin. Invest. **51**, 473 (1972).
SLINGERLAND, D.W., BURROWS, B.A.: A probable abnormality in intrathyroidal iodine metabolism in hyperthyroidism. J. clin. Endocr. **22**, 368 (1962).
STANBURY, J.B., JANSSEN, M.A.: The iodinated albumin-like component of the plasma of thyrotoxic patients. J. clin. Endocr. **22**, 978 (1962).
WARTOFSKY, L., INGBAR, S.H.: Estimation of the rate of release of non-thyroxine iodine from the thyroid glands of normal subjects and patients with thyrotoxicosis. J. clin. Endocr. **33**, 488 (1971).
WERNER, S.C.: Classification of thyroid disease. J. clin. Endocr. **29**, 860 (1969).
WOEBER, K.A., SOBEL, R.J., INGBAR, S.H., STERLING, K.: The peripheral metabolism of triiodothyronine in normal subjects and in patients with hyperthyroidism. J. clin. Invest. **49**, 643 (1970).

Graves' Disease

Incidence

FURSZYFER, J., KURLAND, L.T., MCCONAHEY, W.M., ELVEBACK, L.R.: Graves' disease in Olmsted County, Minnesota, 1935 through 1967. Mayo Clin. Proc. **45**, 636 (1970).
— — — WOOLNER, L.B., ELVEBACK, L.R.: Epidemiologic aspects of Hashimoto's thyroiditis and Graves' disease in Rochester, Minnesota (1935–1967), with special reference to temporal trends. Metabolism **21**, 197 (1972).
GREENWALD, I.: Epidemiologic aspects of thyrotoxicosis. Acta endocr. (Kbh.) **51**, 57 (1966).
LAMBERG, B.A., GORDIN, A., VIHERKOSKI, M., KVIST, G.: Long acting thyroid stimulator (LATS) in toxic nodular goiter, toxic adenoma and Graves' disease. Acta endocr. (Kbh.) **62**, 199 (1969).

RONNOV-JESSEN, V., KIRKEGARD, C.: Hyperthyroidism—A disease of old age? Brit. med. J. **1973 I**, 41.

Etiology and Pathogenesis, LATS Familial Incidence

ADAMS, D.D.: Pathogenesis of the hyperthyroidism of Graves' disease. Brit. med. J. **1965 I**, 1015.
— The presence of an abnormal thyroid-stimulating hormone in the serum of some thyrotoxic patients. J. clin. Endocr. **18**, 699 (1958).
— KENNEDY, T.H.: Occurrence in thyrotoxicosis of a gamma-globulin which protects LATS from neutralisation by an extract of thyroid gland. J. clin. Endocr. **27**, 173 (1967).
— — Evidence to suggest that LATS protector stimulates the human thyroid gland. J. clin. Endocr. **33**, 47 (1971).
— PURVES, H.D.: The role of thyrotropin in hyperthyroidism and exophthalmos. Metabolism **6**, 26 (1957).
ALEXANDER, W.D., KOUTRAS, D.A., BROOKS, J., BUCHANAN, W.W., MACDONALD, E.M., RICHMOND, M.H., WAYNE, E.J.: Quantitative studies of iodine metabolism in thyroid disease. Quart. J. med. **31**, 281 (1962).
AMINO, N., MIYAI, K., AZUKIZAWA, M., KUMAHAR, Y.: Studies on the interaction of long-acting thyroid stimulator (LATS) with soluble thyroid fractions. Acta endocr. (Kbh.) **68**, 625 (1971).
ARNAUD, C.D., KNEUBUHLER, H.A., SEILING, V.L., WIGHTMAN, B.K., ENGBRING, N.H.: Responses of the normal human to infusions of plasma from patients with Graves' disease. J. clin. Invest. **44**, 1287 (1965).
BARTELS, E.D.: Heredity in Graves' disease. Copenhagen: Ejnar Munksgaards Forlag 1941.
BEALL, G.N., SOLOMON, D.H.: On the immunological nature of the long acting thyroid stimulator. J. clin. Endocr. **26**, 1328 (1966).
— — Thyroid-stimulating activity in the serum of rabbits immunized with thyroid microsomes. J. clin. Endocr. **28**, 503 (1968).
BECKERS, D.V.: Effects of hypophysectomy on certain parameters of thyroid function in two patients with Graves' disease. J. clin. Endocr. **19**, 840 (1959).
BENHAMOU-GLYNN, N., EL KABIR, D.J., ROITT, J.M., DONIACH, D.: Studies on the antigen reacting with the thyroid-stimulating immunoglobulin (LATS) in thyrotoxicosis. Immunology **16**, 187 (1969).
BENHAMOU-GLYNN, N., EL KABIR, J., DONIACH, D., ROITT, I.M.: Inhibition of the thyroid-stimulating globulin (LATS) by particulate and soluble cell fractions. Proc. roy. Soc. Med. **61**, 1303 (1968).
— — ROITT, I.M., DONIACH, D.: Studies on the antigen reacting with the thyroid-stimulating immuno-globulin (LATS) in thyrotoxicosis. Immunology **16**, 187 (1969).
BOAS, N.F., OBER, W.B.: Hereditary exophthalmic goiter-report of eleven cases in one family. J. clin. Endocr. **6**, 575 (1946).
BONNYNS, M., DEMEESTER-MIRKINE, N., CALAY, R., BASTENIE, T.A.: Evaluation of the relationship between long-acting thyroid stimulator, clinical and biological thyrotoxicosis and exophthalmus. Acta endocr. (Kbh.) **58**, 581 (1968).
BRODY, J.I., GREENBERG, S.: Lymphocyte dysfunction in thyrotoxicosis. J. clin. Endocr. **35**, 574 (1972).
BROWN, J., ENSOR, J., MUNRO, D.S.: A comparison of the actions of thyroid stimulating hormone and the long-acting thyroid stimulator in an *in vitro* assay. Proc. roy. Soc. Med. **61**, 652 (1968).
BURKE, G.: On the interaction of long acting thyroid stimulator with thyroid microsomes. J. clin. Endocr. **27**, 1095 (1967).
— On the competitive interaction of long-acting thyroid stimulator and thyrotropin *in vivo*. J. clin. Endocr. **28**, 286 (1968).
— The long-acting thyroid stimulator of Graves' disease. Amer. J. Med. **45**, 435 (1968).
— Thyroid stimulators and thyroid stimulation. Acta Endocr. (Kbh.) **66**, 538 (1971).
CARNEIRO, L., DORINGTON, K.J., MUNRO, D.S.: Recovery of the long-acting thyroid stimulator from serum of patients with thyrotoxicosis by concentration of immunoglobulin G. Clin. Sci. **31**, 215 (1966).
CHERTOW, B.C., FIDLER, W.J., FARISS, B.L.: Graves' disease and Hashimoto thyroiditis in monozygous twins. Acta endocr. (Kbh.) **72**, 18 (1973).
CHOPRA, I.J., BEALL, G.N., SOLOMON, D.H.: LATS inhibition by a soluble lipoprotein from human thyroid. J. clin. Endocr. **32**, 772 (1971).
— SOLOMON, D.H., JOHNSON, D.E., CHOPRA, U.: Thyroid gland in Graves' disease: victim or culprit? Metabolism **19**, 760 (1970).
DONIACH, D., ROITT, I.M., BENHAMON-GLYNN, N., JAYSON, M.I.V., EL KABIR, D.J.: Thyrotoxicosis and non-toxic Hashimoto goiter in monozygotic twins. In: IRVINE, W.J., Thyrotoxicosis. Edinburgh-London: Livingstone 1967.
— — TAYLOR, K.B.: Autoimmune phenomena in pernicious anaemia, serological overlap with thyroiditis, thyrotoxicosis and systemic lupus erythematodus. Brit. med. J. **1963 I**, 1374.
Editorial: Inheritance of hyperthyroidism. Brit. med. J. **1973 II**, 5.
EIKHOFF, W.: Schilddrüse und Basedow. Stuttgart: Thieme 1949.
FAJANS, S.S.: Hyperthyroidism in a patient with post-partum necrosis of the pituitary: Case report and implications. J. clin. Endocr. **18**, 271 (1958).
FARID, N.R., MUNRO, R.E., ROW, V.V., VOLPÉ, R.: Peripheral thymis-dependant (T)lymphocytes in Graves' disease and Hashimoto's thyroiditis. New Engl. J. Med. **288**, 1313 (1973).
FATOURECHI, V., MCCONAHEY, W.M., WOOLNER, L.B.: Hyperthyroidism associated with histologic Hashimoto's thyroiditis. Mayo Clin. Proc. **46**, 682 (1971).
FLORSHEIM, W.H., WILLIAMS, A.D., SCHONBAUM, E.: On the mechanism of the MCKENZIE bioassay. Endocrinology **87**, 881 (1970).
GARRY, R., HALL, R.: Stimulation of mitosis in rat thyroid by long-acting thyroid stimulator. Lancet **1970 I**, 693.
HALES, I.B., DOBYNS, B.M.: The metabolism of triiodothyronine in Graves' disease. J. clin. Endocr. **20**, 68 (1960).
HALL, R.: Hyperthyroidism. Pathogenesis and diagnosis. Brit. med. J. **1970 I**, 743.
HASSAN, T.H.A., GREIG, W.R., BOYLE, J.A., BOYLE, I.T., WALLACE, T.J.: Toxic diffuse goitre in monozygotic twins. Lancet **1966 II**, 306.
HETZEL, E.S.: The etiology and pathogenesis of hyperthyroidism. Postgrad. med. J. **44**, 363 (1968).
HILTON, J.G., BLACK, W.C., ATHOS, W., MCHUGH, B., WESTERMANN, C.D.: Increased ACTH-like activity in plasma of patients with thyrotoxicosis. J. clin. Endocr. **22**, 900 (1062).
IRVINE, W.J.: Thyroid auto-immunity as a disorder of immunological tolerance. Quart. J. exp. Physiol. **49**, 324 (1964).
JAYSON, M.I.V., DONIACH, D., BENHAMON-GLYNN, N., ROITT, I.M., EL KABIR, D.J.: Thyrotoxicosis and Hashimoto goitre in a pair of monozygotic twins with serum long-acting thyroid stimulator. Lancet **1967 II**, 15.
KANEKO, T., ZOR, U., FIELD, J.B.: Stimulation of thyroid adenyl cyclase activity and cyclic adenosine 3',5'-monophosphate by long-acting thyroid stimulator. Metabolism **19**, 430 (1970).
KRISS, J.P.: Inactivation of long-acting thyroid stimulator (LATS) by anti-kappa and anti-lambda antisera. J. clin. Endocr. **28**, 1440 (1968).
— PLESHAKOW, V., CHIEN, J.R., ROSENBLUM, A.: Studies on the formation of long-acting thyroid globulin (LATS)

and the alteration of its biologic activity by enzymatic digestion and partial chemical degradation in Current topics in thyroid research. New York-London: Academic Press 1965.

— Pleshakov, V., Chien, J.R.: Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial myxedema. J. clin. Endocr. **24**, 1005 (1964).

— — Rosenblum, A.L., Holderness, M., Sharp, G., Utiger, R.: Studies on the pathogenesis of the ophthalmopathy in Graves' disease. J. clin. Endocr. **27**, 582 (1967).

Lamki, L., Row, V.V., Volpé, R.: Cell-mediated immunity in Graves' disease and in Hashimoto's thyroiditis as shown by the demonstration of migration inhibition factor (MIF). J. clin. Endocr. **36**, 358 (1973).

Leading Article: Placental transmission of autoimmune diseases. Brit. med. J. **1966 I**, 1554.

Levitt, T.: The thyroid. Edingbourgh-London: Livingstone 1954.

Liddle, G.W., Heyssel, R.M., McKenzie, J.M.: Graves' disease without hyperthyroidism. Amer. J. Med. **39**, 845 (1965).

Lipman, L.M., Green, D.E., Snyder, N.J., Nelson, J.C., Solomon, D.H.: Relationship of long-acting thyroid stimulator to the clinical features and course of Graves' disease. Amer. J. Med. **43**, 486 (1967).

Mahaux, J.E., Delcourt, R., Chamla-Soumenkoff, J., Nagel, M.: Aspect autoimmunitaire du goître exophthalmique. Activation des structures lymphoïdes cervicales et production d'immunoglobulines IgM et IgG. Ann. Endocr. (Paris) **30**, 139 (1969).

Martin, L., Fisher, R.A.: The hereditary and familial aspects of toxic nodular goiter (secondary thyrotoxicosis). Quart. J. Med. **20**, 293 (1951).

Major, P.W., Munro, D.S.: Observations on the stimulation of thyroid function in mice by the injection of serum from normal subjects and from patients with thyroid disorder. Clin. Sci. **23**, 463 (1962).

McKenzie, J.M.: Neonatal Graves' disease. J. clin. Endocr. **24**, 660 (1964).

— The long-acting thyroid stimulator: its role in Graves' disease. Recent Progr. Hormone Res. **23**, 1 (1967).

— Humoral factors in the pathogenesis of Graves' disease. Physiol. Rev. **48**, 252 (1968).

— Experimental production of a thyroid-stimulating antithyroid antibody. J. clin. Endocr. **28**, 596 (1968).

— Does LATS cause hyperthyroidism in Graves' disease? (A review biased toward the affirmative). Metabolism **21**, 883 (1972).

— McCullagh, E.P.: Observations against a causal relationship between the long-acting thyroid stimulator and ophthalmopathy in Graves' disease. J. clin. Endocr. **28**, 1077 (1968).

— Solomon, S.H.: Neuroendocrine factors in thyroid disease. In: An introduction to clinical neuroendocrinology, E. Bajus (ed.) New York: Karger 1967.

Miyai, K., Werner, S.C.: Concentration of long-acting thyroid stimulator (LATS) by subfractonation of γG-globulin from Graves' disease serum J. clin. Endocr. **26**, 504 (1966).

Mori, T., Kriss, J.P.: Measurements by competitive binding radioassay of serum antimicrosomal and antithyroglobulin antibodies in Graves' disease and other thyroid disorders. J. clin. Endocr. **33**, 688 (1971).

Noguchi, A., Kurikaro, H., Sato, S.: Clinical studies on the long-acting thyroid stimulator. J. clin. Endocr. **24**, 160 (1964).

Notter, G.: Influence of ACTH on the accumulation of radioiodine in the human thyroid—a new extra-adrenocortical effect of ACTH. J. clin. Endocr. **22**, 817 (1962).

Oberdisse, K., Klein, E.: Die Krankheiten der Schilddrüse. (p. 179, zum sog. Schreckbasedow). Stuttgart: Thieme 1967.

Ochi, Y., De Groot, L.J.: Current concepts: The long acting thyroid stimulator of Graves' disease. New Engl. J. Med. **278**, 718 (1968).

— — Stimulation of thyroid hyperplasia and protein synthesis by LATS. Endocrinology **85**, 344 (1969).

Patel, Y.C., Burger, M.G., Hudson, B.: Radioimmunoassay of serum thyrotropin: sensitivity and specificity. J. clin. Endocr. **33**, 768 (1971).

Pazianos, A.G., Benua, R., Ray, B.S., Pearson, O.H.: Persistent thyroid function following hypophysectomy. J. clin. Endocr. **20**, 1051 (1960).

Plimstone, B.L., Hoffenberg, R., Black, E.: Parallel assays of thyrotropin, long-acting thyroid stimulator and exophthalmos-producing substance in some endocrine disorders. J. clin. Endocr. **23**, 236 (1963).

Pinchera, A., Pinchera, M.G., Stanbury, J.B.: Thyrotropin and long-acting thyroid stimulator assays in thyroid disease. J. clin. Endocr. **25**, 189 (1965).

Pinchera, A., Liberti, P., Martino, E., Fenzi, G.F., Grasso, L., Rovis, L., Baschieri, L., Doria, G.: Effects of antithyroid therapy on the long-acting thyroid stimulator and the antithyroglobulin antibodies. J. clin. Endocr. **29**, 231 (1969).

— Rovis, L., Grasso, L., Liberti, P., Martino, E., Fenzi, G.F., Baschieri, L.: Radioimmunological studies on the long-acting thyroid stimulator. Endocrinology **87**, 217 (1970).

Sato, S., Noguchi, S., Noguchi, A.: Purification and characterization of long-acting thyroid stimulator inhibiting factor from human thyroid tissue. Biochim. biophys. Acta (Amst.) **273**, 299 (1972).

Scazziga, B.R., Lemarchand-Béraud, Th.: Le rôle de l'hypophyse en pathologie thyroïdienne. Schweiz. med. Wschr. **98**, 148 (1968).

Schäfer, H., Voss, C., Nenschel, H.J., Hartmann, N.: Jodtyrosin-Dejodasen im Stoffwechsel der Schildrüsen-Hormone („Schreck-Basedow"). Hoppe Seylers Z. physiol. Chem. **341**, 268 (1965).

Schermer, D.R., Roenik, H.H., Schumacher, O.P., McKenzie, J.M.: Relationship of long-acting thyroid stimulator to pretibial myxedema. Arch. Derm. **102**, 62 (1970).

Schleusener, H., Murthy, P.V.N., McKenzie, J.M.: Studies on the thyroid gland component inhibiting LATS. Metabolism **20**, 299 (1971).

Sellers, E.A., Awad, A.G., Schönbaum, E.: Long-acting thyroid stimulator in Graves' disease. Lancet **1970 II**, 335.

Shishiba, Y., Shimizu, T., Yoshimura, S., Shizume, K.: Direct evidence for human thyroidal stimulation by LATS protector. J. clin. Endocr. **36**, 517 (1973).

Silverstein, G.E., Burke, G.: Thyroid suppressibility and long-acting thyroid stimulator in thyrotoxicosis. Arch. intern. Med. **126**, 615 (1970).

Skillern, P.G.: Genetics of Graves' disease. Mayo Clin. Proc. **47**, 848 (1972).

Smith, B.R., Munro, D.S.: The nature of the interaction between thyroid stimulating γ-G-globulin (long-acting thyroid stimulator) and thyroid tissue. Biochim. biophys. Acta (Amst.) **208**, 285 (1970).

Solomon, D.H., Beall, G.N.: Thyroid stimulating activity in the serum of immunized rabbits. II. Nature of the thyroid stimulating material. J. clin. Endocr. **28**, 1496 (1968).

— — Chopra, I.J.: Stimulation of release of thyroid iodine by serum of thyroid-immunized rabbits. J. clin. Endocr. **31**, 603 (1970).

Studer, H.: Hyperthyreose and "long acting thyroid stimulator" (LATS). Schweiz. med. Wschr. **97**, 622 (1967).

Sunshine, P., Kusumoto, H., Kriss, J.P.: Survival time of circulating long-acting thyroid stimulator in neonatal thyrotoxicosis. Pediatrics **36**, 869 (1965).

Taunton, O.D., Pittman, J.A., Jr.: Hyperthyroidism following secondary hypothyroidism. J. clin. Endocr. **24**, 934 (1964).

UTHGENANNT, H., MÜLLER, W., WEINREICH, J.: Über Häufigkeit und klinische Bedeutung von Autoantikörpern bei Schilddrüsenerkrankungen, insbesondere bei der Hyperthyreose. Dtsch. med. Wschr. **91**, 437 (1965).
VOLPÉ, R., EDMONDS, M., LAMKI, L., CLARK, P.V., ROW, V.V.: The pathogenesis of Graves' disease. A disorder of delayed hypersensitivity? Mayo Clin. Proc. **47**, 824 (1972).
WALL, J.R., GOOD, B.F., HETZEL, B.S.: Long-acting thyroid stimulator in euthyroid relatives of thyrotoxic patients. Lancet **1969 II**, 1024.
WEISBECKER, L., UTHGENANNT, H., SCHEMMEL, K., MÜLLER, W., HEESEN, H., EICKENBUSCH, W., BINDEBALLE, W.: Long-acting thyroid stimulator (LATS) bei Schilddrüsenerkrankungen und seine Beziehung zu den Schilddrüsenantikörpern. Schweiz. med. Wschr. **97**, 898 (1967).
WERNER, S.C., WEGELIUS, O., FIERER, J.A., HSU, K.C.: Immunoglobulins (E, M, G) and complement in thyroid of Graves' disease. New Engl. J. Med. **287**, 421 (1972).

Pathology of Thyroid Gland in Hyperthyroidism

OLEN, E., KLINCK, G.H.: Hyperthyroidism and thyroid cancer. Arch. Path. **81**, 531 (1966).

Cardiovascular System and Lungs

BREWSTER, W.R., JR., ISAACS, J.P., OSGGOD, P.F., KING, T.L.: The hemodynamic and metabolic interrelationships in the activity of epinephrine, norepinephrine and the thyroid hormones. Circulation **13**, 1 (1956).
BUCCINO, R.A., SPANN, J.F., JR., POOL, P.E., SONNENBLICK, E.H., BRAUNWALD, E.: Influence of the thyroid state on the intrinsic contractile properties and energy stores of the myocardium. J. clin. Invest. **46**, 1669 (1967).
DE GROOT, W.J., LEONARD, J.J.: Hyperthyroidism as a high cardiac output state. Amer. Heart J. **79**, 265 (1970).
— — PALEY, H.W., JOHNSON, J.E., WARREN, J.V.: The importance of autonomic integrity in maintaining the hyperkinetic circulating dynamics of human hyperthyroidism. J. clin. Invest. **40**, 1033 (1961).
FRYE, R.L., BRAUNWALD, D.E.: Studies on digitalis. III. The influence of triiodothyronine on digitalis requirements. Circulation **23**, 376 (1961).
GRAETTINGER, J.S., MUENSTER, J.J., SILVERSTONE, L.A., CAMPBELL, J.A.: A correlation of clinical and hemodynamic studies in patients with hyperthyroidism with and without congestive heart failure. J. clin. Invest. **38**, 1316 (1959).
GROSSMAN, W., ROBIN, N.J., JOHNSON, L.W., BROOKS, H.L., SELENKOW, H.A., DEXTER, L.: The enhanced myocardial contractility of thyrotoxicosis. Ann. intern. Med. **74**, 869 (1971).
HOWITT, G., ROWLANDS, D.J.: The heart in hyperthyroidism. Amer. Heart J. **73**, 282 (1967).
KONTOS, A.H.: Mechanism of certain abnormalities of the circulation to the limbs in thyrotoxicosis. J. clin. Invest. **44**, 947 (1965).
MASSEY, D.G., BECKLAKE, M.R., MCKENZIE, J.M., BATES, D.V.: Circulatory and ventilatory response to exercise in thyrotoxicosis. New Engl. J. Med. **276**, 1104 (1967).
MORROW, D.H., GAFFNEY, T.E., BRAUNWALD, E.: Studies on digitalis. VII. Influence of hyper- and hypothyroidism on the myocardial response to ouabain. J. Pharmacol. exp. Ther. **140**, 324 (1963).
ROWE, G.G., HUSTON, J.H., WEINSTEIN, A.B., TUCHMAN, H., BROWN, J.F., CRUMPTON, C.W.: The hemodynamics of thyrotoxicosis in man with special reference to coronary blood flow and myocardial oxygen metabolism. J. clin. Invest. **35**, 272 (1956).
SANDLER, G., WILSON, G.M.: The nature and prognosis of heart disease in thyrotoxicosis. Quart. J. Med. **28**, 347 (1959).
SCHOOT, J.B. VAN DER, MORAN, N.C.: An experimental evaluation of the reputed influence of thyroxine on the cardiovascular effects of catecholamines. J. Pharmacol. exp. Ther. **149**, 336 (1965).
STEIN, M., KIMBEL, P., JOHNSON, R.L.: Pulmonary function in hyperthyroidism. J. clin. Invest. **40**, 348 (1961).
SUMMERS, V.K., SURTEES, S.J.: Thyrotoxicosis and heart disease. Acta med. scand. **169**, 661 (1961).
UEHLINGER, A., SCHAUB, F., BÜHLMANN, A.: Die Kreislaufanpassung bei Hyperthyreose. Untersuchungen mittels Herzkatheterismus. Klin. Wschr. **40**, 130 (1962).
WILSON, W.R., THEILEN, E.O., FLETCHER, F.W.: Pharmacodynamic effects of β-adrenergic receptor blockade in patients with hyperthyroidism. J. clin. Invest. **43**, 1697 (1964).
— — HEGE, J.H., VALENCA, M.R.: Effects of beta-adrenergic receptor blockade in normal subjects before, during, and after triiodothyronine-induced hypermetabolism. J. clin. Invest. **45**, 1159 (1966).

Neuromuscular System

ADAMS, R.D., DENNY-BROWN, D., PEARSON, C.M.: Diseases of muscle. A study in pathology. New York: Harper and Row 1967.
BOSTRÖM, H., HED, R.: Thyrotoxic myopathy and polymyositis in elderly patients: Differential-diagnostic viewpoints. Acta med. scand. **162**, 225 (1958).
EAYRS, J.T.: Influence of the thyroid on the central nervous system. Brit. med. Bull. **16**, 122 (1960).
ENGEL, A.G.: Thyroid function and periodic paralysis. Amer. J. Med. **30**, 327 (1961).
— Neuromuscular manifestations of Graves' disease. Mayo Clin. Proc. **47**, 919–924 (1972).
ERNSTER, L., IKKOS, D., LUFT, R.: Enzymatic activites of human muscle mitrochondria: a tool in metabolic research. Nature (Lond.) **184**, 1851 (1959).
HAVARD, C.W.H., CAMPBELL, E.D.R., ROSS, H.B., SPENCE, A.W.: Electromyographic and histologic findings in muscles of patients with thyrotoxicosis. Quart. J. Med. **32**, 145 (1963).
LUDIN, H.P., SPIESS, H., KOENIG, M.P.: Neuromuscular dysfunction associated with thyrotoxicosis. Eur. Neurol. **2**, 269 (1969).
MCCOMAS, A.J.: Neuropathy in thyrotoxicosis. New Engl. J. Med. **289**, 219 (1973).
MCFADZEAN, A.J.S., YEUNG, R.: Periodic paralysis complicating thyrotoxicosis in Chinese. Brit. med. J. **1967 I**, 451.
MILLIKAN, C., HAINES, S.F.: The thyroid gland in relation to neuromuscular disease. Arch. intern. Med. **92**, 5 (1953).
NOSEDA, G.: Die chronische Myopathie bei Schilddrüsenüberfunktion. Schweiz. med. Wschr. **97**, 797, 842 (1967).
OHINAKA, S., SHIZUME, K., INO, S., WATANABE, A., IRIE, M., NOGUCHI, A., KUMA, S., KUMA, K., IFO, T.: The association of periodic paralysis and hyperthyroidism in Japan. J. clin. Endocr. **17**, 1454 (1957).
OKIHIRO, M.M., BEDDOW, R.M.: Thyrotoxic periodic paralysis in Hawaii. Its predilection for the Japanese race. Neurology (Minneap.) **15**, 253 (1965).
RAMSAY, I.D.: Muscle dysfunction in hyperthyroidism. Lancet **1966 II**, 931.
— Thyrotoxic muscle disease. Postgrad. med. J. **44**, 385 (1968).
RESNICK, J.S., DORMAN, J.D., ENGEL, W.K.: Thyrotoxic periodic paralysis. Amer. J. Med. **47**, 831 (1969).
SHIZUME, K., SHISHIBA, Y., SAKUMA, M., YAMANCHI, H., NAKAO, K., OKINAKA, S.: Studies on electrolyte metabolism in idiopathic and thyrotoxic periodic paralysis. Metabolism **15**, 138 (1966).
SIERSBAEK-NIELSON, K., HANSEN, J.M., SCHIOLER, M., KRISTENSEN, M., STOIER, M., OLSEN, P.Z.: Electroencephalographic changes during and after treatment of hyperthyroidism. Acta endocr. (Kbh.) **70**, 308 (1972).

SOKOLOFF, L., WECHSLER, R.L., BALLS, K., KETY, S.: The relation of the cerebral O_2 consumption to the total body metabolism in hyperthyroidism. J. clin. Invest. **29**, 847 (1950).
STOCKER, W.W., SAMAHA, F.J., DE GROOT, L.J.: Coupled oxidative phosphorylation in muscle of thyrotoxic patients. Amer. J. Med. **44**, 900 (1968).
WHITFIELD, A.G.W., HUDSON, W.A.: Chronic thyrotoxic myopathy. Quart. J. Med. **30**, 257 (1961).

Bones, Joints and Calcium Metabolism

ASKANAZY, M., RUTISHAUSER, E.: Die Knochen des Basedowkranken. Virchows Arch. path. Anat. **291**, 653 (1933).
BAXTER, J.D., BONDY, P.K.: Hypercalcemia of thyrotoxicosis. Ann. intern. Med. **65**, 429 (1966).
FOLLIS, H.R., JR.: Skeletal changes associated with hyperthyroidism. Bull. Johns Hopkins Hosp. **92**, 405 (1953).
FRASER, S.A., ANDERSON, J.B., SMITH, D.A., WILSON, G.M.: Osteoporosis and fractures following thyrotoxicosis. Lancet **1971 I**, 981.
GIMLETTE, T.M.D.: Thyroid acropachy. Lancet **1960 I**, 22.
HARDEN, R.M., HARRISON, M.T., ALEXANDER, W.D., NORDIN, B.E.C.: Phosphate excretion and parathyroid function in thyrotoxicosis. J. Endocr. **28**, 281 (1964).
HARPER, P.S., HUGHES, R.O.: Severe hypercalcemia from hyperthyroidism with unusual features. Brit. med. J. **1970 I**, 213.
JALLUT, O., LABHART, A., UEHLINGER, E., KÖNIG, M.P.: Akropachie bei thyreo-hypophysärem Syndrom. Schweiz. med. Wschr. **92**, 255 (1962).
KLEIN, E.: Die thyreogenen Osteopathien. Dtsch. med. Wschr. **21**, 1087 (1963).
KÖNIG, M.P., GUBLER, R.: Hypercalcämie bei Hyperthyreose. Schweiz. med. Wschr. **89**, 369 (1959).
KRANE, S.M., BROWNELL, G.L., STANBURY, J.B., CORRIGAN, H.: The effect of thyroid disease on calcium metabolism in man. J. clin. Invest. **35**, 874 (1956).
MEGLIOLI, G.T.: Osteopathie bei Hyperthyreose. Schweiz. med. Wschr. **96**, 647 (1966).
MICHIE, W., STOWERS, J.M., DUNCAN, T., PEGG, C.A.S., HAMER-HODGES, D.W., HEMS, G., BEWSHER, P.D., HEDLEY, A.J.: Mechanism of hypocalcemia after thyroidectomy for thyrotoxicosis. Lancet **1971 I**, 508.
NIXON, D.W., SAMOLS, E.: Acral changes associated with thyroid diseases. J. Amer. med. Ass. **212**, 1175 (1970).
PARFITT, A.M., DENT, C.E.: Hyperthyroidism and hypercalcaemia. Quart. J. Med. **39**, 171 (1970).
SATALINE, L.E., POWEL, CH., HAMWI, G.J.: Suppression of the hypercalcemia of thyrotoxicosis by corticosteroids. New Engl. J. Med. **267**, 646 (1962).
TWYCROSS, R.G., MARKS, V.: Symptomatic hypercalcemia in thyrotoxicosis. Brit. med. J. **1970 II**, 701.

Skin

HALI, R., HOLLI, G., STEVENSON, J.C.: Treatment of localized myxoedema. Brit. med. J. **1965 II**, 1368.
KIRKEBY, K., HANGAARD, G., LINGJAERDE, P.: The pigmentation of thyrotoxic patients. Acta med. scand. **174**, 257 (1963).
KRISS, J.P., PLESHAKOV, V., CHIEN, J.R.: Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial myxedema. J. clin. Endocr. **24**, 1005 (1964).
— — ROSENBLUM, A., SHARP, G.: Therapy with occlusive dressings of pretibial myxedema with fluocinolone acetonide. J. clin. Endocr. **27**, 595, 1967.
OCHI, Y., DE GROOT, L.J.: Vitiligo in Graves' disease. Ann. intern. Med. **71**, 935 (1969).
SISSON, J.C.: Hyaluronic acid in localized myxedema. J. clin. Endocr. **28**, 433 (1968).

Gastrointestinal Tract, Liver

BAKER, J.T., HARVEY, R.F.: Bowel habit in thyrotoxicosis and hypothyroidism. Brit. med. J. **1971 I**, 322.
BOCK, O.A.A., WITT, L.J.: Gastric acidity and gastric biopsy in thyrotoxicosis. Brit. med. J. **1963 II**, 20.
CRANE, C.W., EVANS, D.W.: Thyrotoxic steatorrhoea. Brit. med. J. **1966 II**, 1575.
GREENBERGER, N.J., MILLIGAN, F.D., GROOT, L.J. DE, ISSELBACHER, K.: Jaundice and thyrotoxicosis in the absence of congestive heart failure. Amer. J. Med. **36**, 840 (1964).
KLION, F.M., SEGAL, R., SCHAFFNER, F.: The effect of altered thyroid function on the ultrastructure of the human liver. Amer. J. Med. **50**, 317 (1971).
LAMBERG, B.A., GORDIN, R.: Liver function in thyrotoxicosis. Acta endocr. (Kbh.) **15**, 82 (1954).
MOVITT, E.R., GERSTL, B., DAVIS, A.E.: Needle liver biopsy in thyrotoxicosis. Arch. intern. Med. **91**, 729 (1953).
SIURALA, M., LAMBERG, B.A.: Stomach in thyrotoxicosis. Acta med. scand. **165**, 181 (1959).
THOMAS, F.B., CALDWELL, J.H., GREENBERGER, N.J.: Steatorrhea in thyrotoxicosis. Ann. intern. Med. **78**, 669 (1973).
WEBER, S.: Leberfunktionsstörungen bei Hyperthyreose. Praxis **57**, 2 (1968).

Lipid and Carbohydrate Metabolism

DIETERLE, P., BOTTERMANN, P., LANDGRAF, R., SCHWARZ, K., SCRIBA, P.C.: Der Kohlenhydratstoffwechsel bei Schilddrüsenfunktionsstörungen. Med. Klin. **64**, 489 (1969).
JONES, J.E., DESPER, P.C., SHANE, S.R., FLINK, E.B.: Magnesium metabolism in hyperthyroidism and hypothyroidism. J. clin. Invest. **45**, 891 (1966).
KOSAK, G.P.: Diabetes and other endocrinologic disorders. In: Joslin's Diabetes mellitus (A. MARBLE *et al.*, eds.), p. 666. Philadelphia: Lea & Febiger 1971.
LAMBERG, B.A.: Glucose metabolism in thyroid disease. Acta med. scand. **178**, 351 (1965).
NIKKILÄ, E.A., KEKKI, M.: Plasma triglyceride metabolism in thyroid disease. J. clin. Invest. **51**, 2102 (1972).

Blood

ARDEMAN, S., CHANARIN, I., KRAFCHIK, B., SINGER, W.: Addisonian pernicious anaemia and intrinsic factor antibodies in thyroid disorders. Quart. J. Med., N. S. **35**, 421 (1966).
FURSZYFER, J., MCCONAHEY, W.M., KURLAND, L.T., MALDONADO, J.E.: On the increased association of Graves' disease with pernicious anemia. Mayo Clin. Proc. **46**, 37 (1971).
IVY, H.K.: Severe hypoproteinemia: unusual manifestations of thyrotoxicosis. Arch. intern. Med. **111**, 607 (1963).
KEKKI, M.: Serum protein turnover in experimental hypo- and hyperthyroidism. Acta endocr. (Kbh.), Suppl. **91**, 1 (1964).
MAHAUX, J.E., CHAMLA-SOUMENKOFF, J., DELCOURT, R., LEVIN, S.: Painful enlargement of left supratrapezoid lymph nodes in Graves' disease. Brit. med. J. **1971 I**, 384.
MICHIE, W., BECK, J., MAHAFFY, R.G., HONEIN, E.F., FOWLER, G.: Quantitative radiological and histological studies of the thymus in thyroid disease. Lancet **1967 I**, 691.
PINCHERLE, G.: Case of thyrotoxicosis presenting as hypoproteinaemic oedema. Brit. med. J. **1965 I**, 632.
RIVLIN, R.S., WAGNER, H.N.: Anemia in hyperthyroidism. Ann. intern. Med. **70**, 507 (1969).
SCHILLER, K.F.R., SPRAY, G.H., WANGEL, A.G., WRIGHT, R.: Clinical and precursory forms of pernicious anemia in hyperthyroidism. Quart. J. Med. **147**, 451 (1968).
ULTMANN, J.E., HYMAN, G.A., CALDER, B.: The occurrence of lymphoma in patients with long standing hyperthyroidism. Blood **21**, 282 (1963).

VAGENAKIS, A.G., COTE, R., MILLER, M.E., BRAVERMANN, L.E., STOHLMAN, F.: Enhancement of warfarin-induced hypoprothrombinemia by thyrotoxicosis. Hopkins med. J. **131**, 69 (1972).
WANGEL, A.G., SCHILLER, K.F.R.: Diagnostic significance of antibody to intrinsic factor. Brit. med. J. **1966 I**, 1249, 1274.
WILLIAMS, M.J., SCOTT, G.B., BECK, J.S., BLAIR, D.W.: Antigastric antibodies in hyperthyroidism: their relationship to impaired acid secretion. Brit. med. J. **1966 I**, 388.

Kidney

CUTLER, R.E., GLATTE, H., DOWLING, J.T.: Effect of Hyperthyroidism on the renal concentrating mechanism in humans. J. clin. Endocr. **27**, 453 (1967).
EPSTEIN, F.H., FREEDMAN, L.R., LEVITIN, H.: Hypercalcemia, nephrocalcinosis and reversible renal insufficiency associated with hyperthyroidism. New Engl. J. Med. **258**, 782 (1958).
FORD, R.V., OWENS, J.C., CURD, G.W., JR., MOYER, J.H., SPURR, C.L.: Kidney function in various thyroid states. J. clin. Endocr. **21**, 548 (1961).
HUTH, E.J., MAYOCK, R.L., KERR, R.M.: Hyperthyroidism associated with renal tubular acidosis. Amer. J. Med. **26**, 818 (1959).
LABHART, A., SCHEITLIN, W.: Niere bei Endokrinopathien. In: Handbuch der inneren Medizin, 5. Aufl., Bd. VIII, Teil 3, H. SCHWIEGK (ed.). Berlin-Heidelberg-New York: Springer 1968.

Other Endocrine Glands

ASHKAR, F.S., SMOAK, W.M., GILSON, A.J., MILLER, R.: Gynecomastia and mastoplasia in Graves' disease. Metabolism **19**, 946 (1970).
BEISEL, W.R., DIRAIMONDO, V.C., CHAO, P.Y., ROSNER, J.M., FORSHAM, P.H.: The influence of plasma protein binding on the extraadrenal metabolism of cortisol in normal, in hyperthyroid and hypothyroid subjects. Metabolism **13**, 942 (1964).
BURKE, G., FELDMAN, J.M.: Addison's disease and hyperthyroidism. Amer. J. Med. **38**, 470 (1965).
CHOPRA, I.J., ABRAHAM, G.E., CHOPRA, U., SOLOMON, D.H., ODELL, W.D.: Alterations in circulating estradiol-17β in male patients with Graves' disease. New Engl. J. Med. **286**, 124 (1972).
GALLAGHER, T.F., HELLMAN, L., FINKELSTEIN, J., YOSHIDA, K., WEITZMAN, E.D., ROFFWARG, H.D., FUKUSHIMA, D.K.: Hyperthyroidism and cortisol secretion in man. J. clin. Endocr. **34**, 919 (1972).
GASTINEAU, C.F., MYERS, W.R., ARNOLD, J.W., MCCONAHEY, W.M.: Graves' disease in Addison's disease. Proc. Mayo Clin. **39**, 939 (1965).
KAPPELER, H.J.: Zusammentreffen von Morbus Addison und Hyperthyreose. Helv. med. Acta **32**, 435 (1965).
LARSSON, O., SUNDBOM, C.M., ÅSTEDT, B.: Gynaecomastia and diseases of thyroid. Acta endocr. (Kbh.) **44**, 133 (1963).

Natural Course

MCLARTY, D.C., ALEXANDER, W.D., MCG. HARDEN, R., ROBERTSON, J.W.K.: Self-limiting episodes of recurrent thyrotoxicosis. Lancet **1971 I**, 6.
MURRAY, G.R.: The clinical history and symptoms of 120 cases of exophthalmic goiter. Med. chir. Trans. **86**, 141 (1903).

Differential Diagnosis, Diagnostic Indices

CHAPMAN, M., MALOOF, F.: Bizarre clinical manifestations of hyperthyroidism. New Engl. J. Med. **254**, 1 (1956).
CROOKS, J., MURRAY, I.P.C., WAYNE, E.J.: Statistical methods applied to the clinical diagnosis of thyrotoxicosis. Quart. J. Med. **28**, 211 (1959).
FISCHER, J.A., BOLLINGER, A., LICHTLEN, P., WELLAUER, J.: Fibrous dysplasia of the bone and high cardiac output. Amer. J. Med. **49**, 160 (1970).
GORLIN, R.: The hyperkinetic heart syndrome. J. Amer. med. Ass. **182**, 823 (1962).
GURNEY, C., OWEN, S.G., HALL, R., ROTH, M., HARPER, M., SMART, G.A.: Newcastle thyrotoxicosis index. Lancet **1970 II**, 1275.
HARVEY, R.F.: Indices of thyroid function in thyrotoxicosis. Lancet **1971 II**, 230.
HUBER, A., ESSLEN, E.: Diagnostic des myopathies oculaires à l'aide de électromyographie. Bull. Soc. franç. Ophthal. **80**, 460 (1967).
KRAYENBÜHL, H.: The value of orbital angiography for diagnosis of unilateral exophthalmos. J. Neurosurg. **4**, 289 (1962).
LEON-SOTOMAYOR, L., CHRISTIE, J.N., ZERZAN, CH.J.: Masked thyrotoxicosis. Amer. J. Med. **41**, 473 (1966).
LUFT, R., IKKOS, D., PALMIERI, G., ERNSTER, L., AFZELIUS, B.: A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical and morphological study. J. clin. Invest. **41**, 1776 (1962).
SLOMAN, G.: Propranolol in management of muscular subaortic stenosis. Brit. Heart. J. **29**, 783 (1967).
YASARGIL, M.G.: Die Röntgendiagnostik des Exophthalmus unilateralis. Basel: Karger 1957.

Laboratory Tests

FRAGU, P., THOUIN, A., BAZIN, J.P., TUBIANA, M.: Comparaison de la valeur diagnostique de différents tests fonctionnels thyroïdiens. Intérêt de l'utilisation des fonctions discriminantes. Ann. Endocr. (Paris) **33**, 5 (1972).
GORDON, A., GROVER, N.B., BARZILAI, D., EHRENFELD, E.N., LEWITUS, Z., GROSS, J.: Computer study of thyroid disease. II. Results. Israel J. med. Sci. **6**, 333 (1970).
RIVES, K.L., FURTH, E.D., BECKER, D.V.: Limitations of the ankle jerk test. Intercomparison with other tests of thyroid function. Ann. intern. Med. **62**, 1139 (1965).

Drug Treatment of Graves' Disease

ALEXANDER, W.D., HARDEN, R.MCG., SHIMMINS, J.: Studies of the thyroid iodide "trap" in man. Recent Progr. Hormone Res. **25**, 423 (1969).
— MCHARDEN, R., SHIMMINS, J., MCLARTY, D.G., MCGILL, P.: Treatment of thyrotoxicosis based on thyroidal suppressibility. Lancet **1967 II**, 681.
— MCLARTY, D.G., HORTON, P., PHARMAKIOTIS, A.D.: Sequential assessment during drug treatment of thyrotoxicosis. Clin. Endocr. **2**, 43–50 (1973).
— ROBERTSON, J., SHIMMINS, J., BROWNLIE, B.E., HARDEN, R.MCG., PATEL, A.R.: Prediction of the long-term results of antithyroid drug therapy for thyrotoxicosis. J. clin. Endocr. **30**, 540 (1970).
ASTWOOD, E.B.: Mechanism of action of antithroid compounds. Brookhaven Symposia in Biology No. 7, 61 (1955).
— Use of antithyroid drugs. In: IRVINE, W.J., Thyrotoxicosis. Edinburgh-London: Livingstone 1967.
BARTELS, E.C.: Hyperthyroidism. An evaluation of treatment with antithyroid drugs followed by subtotal thyroidectomy. Ann. intern. Med. **37**, 1123 (1952).

BINSWANGER, CH., STUDER, H., GUBLER, R., WYSS, F.: Does propylthiouracil have extra-thyroidal effects in man? Helv. med. Acta **33**, 197 (1966).
BRAVERMAN, L.E., INGBAR, S.H.: Changes in thyroidal function during adaptation to large doses of iodide. J. clin. Invest. **42**, 1216 (1963).
BÜHLER, U.K., DEGROOT, L.J.: Effect of stable iodine on thyroid iodine release. J. clin. Endocr. **29**, 1546 (1969).
BURKE, G.: Effects of iodide on thyroid stimulation. J. clin. Endocr. **30**, 76 (1970).
CASSIDY, C.E.: Use of thyroid suppression test as a guide to prognosis of hyperthyroidism treated with antithyroid drugs. J. clin. Endocr. **25**, 155 (1965).
— Thyroid suppression test as index of outcome of hyperthyroidism treated with antithyroid drugs. Metabolism **19**, 745 (1970).
CHEVALLEY, J., MCGAVACK, T.H., KENIGSBERG, S., PEARSON, S.: A four-year study of the treatment of hyperthroidism with methimazole, J. clin. Endocr. **14**, 948 (1954).
CHOPRA, I.J., SOLOMON, D.H., JOHNSON, D.E., CHOPRA, U., FISHER, D.: Dissociation of serum LATS content and thyroid suppressibility during treatment of hyperthyroidism. J. clin. Endocr. **30**, 524 (1970).
FEINBERG, W.D., HOFFMAN, D.L., OWEN, C.A.: The effects of varying amounts of stable iodide on the function of the human thyroid. J. clin. Endocr. **19**, 567 (1959).
FISCHER, M.G., NAYER, H.R., MILLER, A.: Methimazole-induced jaundice. J. Amer. med. Ass. **223**, 1028 (1973).
FRIEND, D.G.: Iodide therapy. New Engl. J. Med. **263**, 1358 (1960).
FURTH, E.D., RIVES, K., BECKER, D.V.: Nonthyroidal action of propylthiouracil in euthyroid, hypothyroid and hyperthyroid man. J. clin. Endocr. **26**, 239 (1966).
GALTON, B., PITT-RIVERS, R.: The effect of excessive iodine on the thyroid of the rat. Endocrinology **64**, 835 (1959).
GREER, M.A., MEIHOFF, W.C., STUDER, H.: Treatment of hyperthyroidism with a single daily dose of propylthiouracil. New Engl. J. Med. **272**, 888 (1965).
GODLEY, A.F., STANBURY, J.B.: Treatment of hyperthyroidism with K-perchlorate. J. clin. Endocr. **14**, 70 (1954).
HERRMANN, E.: Die Bedeutung fortgesetzter Thiouracilmedikation für die Proliferation des Schilddrüsengewebes. Schweiz. med. Wschr. **81**, 1097 (1951).
HERSHMAN, J.M., GIVENS, J.R., CASSIDY, C.E., ASTWOOD, E.B.: Long-term outcome of hyperthyroidism treated with antithyroid drugs. J. clin. Endocr. **26**, 803 (1966).
HUNTON, R.B., WELLS, M.V., SKIPPER, E.W.: Hypothyroidism in diabetics treated with sulphonylurea. Lancet **1965 II**, 449.
JOHNSON, R.S., MOORE, G.W.: Fatal aplastic anaemia after treatment of thyrotoxicosis with potassium perchlorate. Brit. med. J. **1961 I**, 1369.
KREVANS, J.R., ASPER, S.P., JR., RIENHOFF, W.F., JR.: Fatal aplastic anemia following use of potassium perchlorate in thyrotoxicosis. J. Amer. med. Ass. **181**, 162 (1962).
LOWRY, R.C., LOWE, D., HADDEN, D.R., MONTGOMERY, D.A.D., WEAVER, J.A.: Thyroid suppressibility: follow-up for two years after antithyroid treatment. Brit. med. J. **1971 II**, 19.
MALOOF, F., SOODAK, M.: Intermediary metabolism of thyroid tissue and the action of drugs. Pharmacol. Rev. **15**, 43 (1963).
MARCHANT, B., ALEXANDER, W.D.: The thyroid accumulation, oxidation and metabolic fate of ^{35}S-methimazole in the rat. Endocrinology **91**, 747 (1972a).
— — LAZARUS, J.H., LEES, J., CLARK, D.H.: The accumulation of ^{35}S-antithyroid drugs by the thyroid gland. J. clin. Endocr. **34**, 847 (1972b).
MCGAVACK, TH.H., CHEVALLEY, J.: Untoward hematologic responses to the antithyroid compounds. Amer. J. Med. **17**, 36 (1954).
MCLARTY, D.G., ALEXANDER, W.D., HARDEN, R.MCG., CLARK, D.H.: Results of treatment of thyrotoxicosis following relapse after antithyroid drug therapy. Brit. med. J. **1969 II**, 203.
— — — — Results of treatment of thyrotoxicosis after postoperative relapse. Brit. med. J. **1969 III**, 200.
MORGANS, M.E., TROTTER, W.R.: Treatment of thyrotoxicosis with potassium perchlorate. Lancet **1954 I**, 749.
MORREALE DE ESCOBAR, G., ESCOBAR DEL REY, F.: Extrathyroidal effects of some antithyroid drugs and their metabolic consequences. Recent Progr. Hormone Res. **23**, 87 (1967).
NAGATAKI, S., SHIZUME, K., NAKAO, K.: Effects of iodide on thyroidal iodine turnover in hyperthyroid subjects. J. clin. Endocr. **30**, 469 (1970).
OPPENHEIMER, J.H., SCHWARTZ, H.L., SURKS, M.I.: Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. An explanation of the antithyroxine effect of propylthiouracil and evidence supporting the concept that triiodothyronine is the active thyroid hormone. J. clin. Invest. **51**, 2493 (1972).
PITTMAN, J.A., BESCHI, R.J., SMITHERMAN, T.C.: Methimazole: Its absorption and excretion in man and tissue distribution in rats. J. clin. Endocr. **33**, 182 (1971).
PLUMMER, H.S.: Results of administering iodine to patients having exophthalmic goiter. J. Amer. med. Ass. **80**, 1955 (1923).
RAWSON, R.W., MOORE, F.D., PEACOCK, W., MEANS, J.H., COPE, O., RIDDELL, C.B.: Effect of iodine on the thyroid gland in Graves' disease when given in conjunction with thiouracil. J. clin. Invest. **24**, 869 (1945).
REINWEIN, D., KLEIN, E.: Der Einfluß des anorganischen Blutjodes auf den Jodumsatz der menschlichen Schilddrüse. Acta endocr. (Kbh.) **35**, 485 (1960).
SHIZUME, K., IRIE, M., NAKATAKI, S., MATSUZAKI, Y., SHISHIBA, V., SUEMATSU, H., TSUSHIMA, T.: Long-term results of antithyroid drug therapy for Graves' disease: follow-up for more than 5 years. Endocr. jap. **17**, 327 (1970).
SLINGERLAND, D.W., BURROWS, B.A.: A probable abnormality in intrathyroidal iodine metabolism in hyperthyroidism. J. clin. Endocr. **22**, 368 (1962).
SOLOMON, D.H., BECK, J.C., VANDERLAAN, W.P., ASTWOOD, E.B.: Prognosis of hyperthyroidism treated by antithyroid drugs. J. Amer. med. Ass. **152**, 201 (1953).
TEMPLE, R., BERMAN, M., CARLSON, H.E., ROBBINS, J., WOLFF, J.: The use of lithium in Graves' disease. Mayo Clin. Proc. **47**, 872 (1972).
VANDERLAAN, W.P.: Antithyroid drugs in practice. Mayo Clin. Proc. **47**, 962 (1972).
— STORRIE, V.M.: A survey of the factors controlling thyroid function, with special reference to newer views on antithyroid substances. Pharmacol. Rev. **7**, 301 (1955).
VAN PILSUM, J.F., BOEN, J.R., BANS, L.: Evidence that the goitrogen methimazole interferes with the extrathyroidal utilization of exogenous thyroxine. Endocrinology **92**, 135 (1973).
WARTOFSKY, L., RANSIL, B.J., INGBAR, S.H.: Inhibition by iodine of the release of thyroxine from the thyroid glands of patients with thyrotoxicosis. J. clin. Invest. **49**, 78 (1970).
WEGELIN, C.: Über den Einfluß des Methylthiouracils auf das histologische Bild der Schilddrüse bei Hyperthyreose. Helv. med. Acta **15**, 3 (1948).
WIBERG, J.J., NUTTALL, F.Q.: Methimazole toxicity from high doses. Ann. intern. Med. **77**, 414 (1972).
WOLFF, J.: Iodide goiter and the pharmacologic effects of excess iodide. Amer. J. Med. **47**, 101 (1969).

Subtotal Thyroidectomy for Graves' Disease

BARTELS, E.C.: Preparation of the hyperthyroid patient for subtotal thyroidectomy. Surg. Clin. N. Amer. **33**, 757 (1953).
BEATUS, O.H., VANDERTOLL, D.J.: Complications of secondary thyroidectomy. Surg. Gynec. Obstet. **117**, 535 (1963).

BUCHANAN, W.W., KOUTRAS, D.A., CROOKS, J., ALEXANDER, W.D., BRASS, W., ANDERSON, J.R., GOUDIE, R.B., GRAY, K.G.: The clinical significance of the complement-fixation test in thyrotoxicosis. J. Endocr. **24**, 115 (1962).

CURRAN, R.C., ECKERT, H., WILSON, G.M.: The thyroid gland after treatment of hyperthyroidism by thyroidectomy or iodine 131. J. Path. Bact. **76**, 541 (1958).

DAVIS, R.H., FOURMAN, P., SMITH, J.W.: Prevalance of parathyroid insufficiency after thyroidectomy. Lancet **1961 II**, 1432.

FOWLER, E.F., DURHAM, W.R., COLE, W.H.: The complications and implications of thyroidectomy. Trans. Am. Goiter Assoc. 1956, p. 463. Springfield: Ch. C. Thomas 1957.

GIRLING, J.A., MURLEY, R.S.: Parathyroid insufficiency after thyroidectomy. Brit. med. J. **1963 I**, 1323.

HARGREAVES, A.W., GARNER, A.: Significance of lymphocytic infiltration of thyroid gland in thyrotoxicosis. Brit. J. Surg. **55**, 543 (1968).

HEDLEY, A.J., FLEMMING, C.J., CHESTERS, M.I., MICHIE, W., CROOKS, J.: Surgical treatment of thyrotoxicosis. Brit. med. J. **1970a I**, 519.

— HALL, R., AMOS, J., MICHIE, W., CROOKS, J.: Serum thyrotropin levels after subtotal thyroidectomy for Graves' disease. Lancet **1970b I**, 455.

— ROSS, I.P., BECK, J.S., DONALD, D., ALBERT-RECHT, F., MICHIE, W., CROOKS, J.: Recurrent thyrotoxicosis after subtotal thyroidectomy. Brit. med. J. **1971 IV**, 258.

MCLARTY, D.G., ALEXANDER, W.D., HARDEN, R.MCG., CLARK, D.H.: Results of treatment of thyrotoxicosis after postoperative relapse. Brit. med. J. **1969 II**, 201.

MCNEIL, A.D., THOMSON, J.A.: Long-term follow-up of surgically treated thyrotoxic patients. Brit. med. J. **1968 III**, 643.

MICHIE, W., PEGG, C.A.S., BEWSHER, P.D.: Prediction of hypothyroidism after partial thyroidectomy for thyrotoxicosis. Brit. med. J. **1972 I**, 13.

— STOWERS, J.M., DUNCAN, T., PEGG, C.A.S., HAMER-HODGES, D.W., HEMS, G., BEWSHER, P.D., HEDLEY, A.J.: Mechanism of hypocalcemia after thyroidectomy for thyrotoxicosis. Lancet **1971 I**, 508.

NOFAL, M.M., BEIERWALTES, W.H., PATUO, M.E.: Treatment of hyperthyroidism with sodium iodide I^{131}. J. Amer. med. Ass. **197**, 605 (1966).

ROY, A.D., QUIE, P.G., MENWISSEN, H.J., HONG, R.: A follow-up of thyrotoxic patients treated by partial thyroidectomy. Lancet **1967 II**, 684.

TAYLOR, G.W., PAINTER, N.S.: Size of the thyroid remnant in partial thyroidectomy for toxic goiter. Lancet **1962 I**, 287.

Radioiodine Treatment of Graves' Disease

ALLEN, H.C., JR., GOODWIN, W.E.: Scintillation counter as instrument for *in vivo* determination of thyroid weight. Radiology **58**, 68 (1952).

ANDREWS, G.A., KINSELEY, R.M., BIGELOW, R.R., ROOT, S.W., BRUCE, B.: Pathologic changes in normal human thyroid tissue following large does of I^{131}. Amer. J. Med. **16**, 372 (1954).

BAKER, H.V.: Anaplastic thyroid cancer 12 years after treatment of thyrotoxicosis with radioiodine. Cancer (Philad.) **22**, 885 (1969).

CANTOLINO, S.J., SCHMICKEL, R.D., BALL, M., CISAR, C.S.: Persistent chromosomal aberrations following radioiodine therapy for thyrotoxicosis. New Engl. J. Med. **275**, 739 (1966).

DAILY, M.E., LINDSAY, S., MILLER, E.R.: Histologic lesions in the thyroid glands of patients receiving radioiodine for hyperthyroidism. J. clin. Endocr. **13**, 1513 (1953).

DOBYNS, B.M., ROBISON, L.R.: Deoxyribonucleic acid content associated with nuclear changes in I^{131}-irradiated human thyroids. J. clin. Endocr. **28**, 875 (1968).

DOERING, P., KMAER, K.: Die Bestimmung des Schilddrüsengewichtes mit der Szintigraphie nach Gabe von Radiojod. Strahlentherapie **105**, 245 (1958).

DUNN, J.T., CHAPMAN, E.M.: Rising incidence of hypothyroidism after radioactive-iodine therapy in thyrotoxicosis. New Engl. J. Med. **271**, 1037 (1964).

Editorial: Radioiodine treatment of thyrotoxicosis. Lancet **1972 I**, 23.

EINHORN, J., WICKLUND, H.: Hypothyroidism following 131-I treatment for hyperthyroidism. J. clin. Endocr. **26**, 33 (1966).

ENGELBRECHT, W.: Zürcher Ergebnisse der Radioresektion bei 698 Patienten mit Morbus Basedow und der Radioelimination bei 404 Patienten mit toxischem Adenom — 1102 Patienten mit Hyperthyreose. Thesis, Zürich, 1970.

FISHER, D.A., PANOS, T.C.: "Due caution" and radioiodine in children. Amer. J. dis. Chid. **103**, 729 (1962).

GAUWERKY, F.: Kritische Übersicht über die Behandlung mit künstlich radioaktiven Isotopen. In: Strahlenbiologie, Strahlentherapie. Nuklearmedizin und Krebsforschung. Stuttgart: Thieme 1959.

GLENNON, J.A., GORDON, E.S., SAWIN, C.T.: Hypothyroidism after low dose ^{131}I treatment of hyperthyroidism. Ann. intern. Med. **76**, 721 (1972).

GOOLDEN, A.W.G., FRASER, T.R.: Treatment of thyrotoxicosis with low doses of radioactive iodine. Brit. med. J. **1969 II**, 442.

GOOLDEN, A.W.G., FRASER, T.R.: Effect of pretreatment with carbimazole in patients with thyrotoxicosis subsequently treated with radioactive iodine. Brit. med. J. **1969 II**, 443.

GREEN, M., WILSON, G.M.: Thyrotoxicosis treated by surgery or iodine-131 with special reference to development of hypothyroidism. Brit. med. J. **1964 I**, 1005.

GREIG, W.R.: Radiation, thyroid cells and I^{131} therapy. A hypothesis. J. clin. Endocr. **25**, 1411 (1965).

HAGEN, G.A., OULETTE, R.P., CHAPMAN, E.M.: Comparison of high and low dosage of I-131 in the treatment of thyrotoxicosis. New Engl. J. Med. **277**, 559 (1967).

HORST, W.: Strahlentherapie der Schilddrüsenerkrankungen mit Radiojod (I^{131}). Internist (Berl.) **8**, 373 (1960).

KAESTNER, F., OTTO, R., ENGELBRECHT, W., HORST, W.: Zur Radioresektion von hyperthyreoten und euthyreoten Strumen und Radioelimination von toxischen Adenomen mit ^{131}Jod. (In preparation.)

KEIDERLING, W.: Therapie der Schilddrüse mit Radiojod. Ergebn. inn. Med. Kinderheilk. **8**, 245 (1957).

LAMBERG, B.A., HERNBERG, C.A., WAHLBERG, P., HAKKILA, R.: Treatment of toxic nodular goiter with radioactive iodine. Acta med. scand. **165**, 245 (1959).

Leading Article: Hypothyroidism after treatment of thyrotoxicosis with radioiodine. Lancet **1965 I**, 637.

LEVENE, M.B., ANDREWS, G.A., KNISELY, R.M.: Large doses of I^{131} in dogs: Radiation dosage correlated with histologic and autoradiographic changes. Amer. J. Roentgenol. **73**, 88 (1955).

LIMA, J.B., CATZ, B., PERJIK, S.L.: Thyroid cancer following ^{131}I therapy of hyperthyroidism. J. nucl. Med. **11**, 46 (1970).

LINDSAY, S., DAILEY, M.E., JONES, M.D.: Histologic effects of various types of ionizing radiation on normal and hyperplastic human thyroid glands. J. clin. Endocr. **14**, 1179 (1954).

MARINELLI, L.D., QUIMBY, E.H., HINE, G.J.: Dosage determination with radioactive isotopes. Amer. J. Roentgenol. **59**, 260 (1948).

MCDOUGALL, I.R., GREIG, W.R., GILLESPIE, F.C.: Radioactive iodine (^{125}I) therapy for thyrotoxicosis. New Engl. J. Med. **285**, 1099 (1971).

— KENNEDY, J.S., THOMPSON, J.A.: Thyroid Carcinoma following I-131 therapy. Report of a case and review of the literature. J. clin. Endocr. **33**, 287 (1971).

MILLER, E.R., LINDSAY, S., DAILEY, M.E.: Studies with radioiodine. V. Validity of histologic determination of J^{131}

radiation. Changes in the thyroid gland. Radiology **65**, 384 (1955).
NOFAL, M.M., BEIERWALTES, W.H., PATNO, M.E.: Treatment of hyperthyroidism with sodium iodide I^{131}. J. Amer. med. Ass. **197**, 605 (1966).
OESER, H., BILLION, H., KÜHNE, P.: Die Behandlung der Hyperthyreose mit Radiojod. In: Künstliche radioaktive Isotope in Physiologie, Diagnostik und Therapie. Berlin-Göttingen-Heidelberg: Springer 1961.
PERRY, C.H.: Internal dose determination of isotopes. A.E.C. Report No. W. 7405-eng-26 (ORNL-1950, 591).
PHILP, J.R., DUTHIE, M.B., CROOKS, J.: A follow-up scheme for detecting hypothyroidism in thyrotoxic patients treated with radioiodine. Lancet **1968 II**, 1336.
POCHIN, E.E.: Leukaemia following radioiodine treatment of thyrotoxicosis. Brit. med. J. **1960 II**, 1545.
SAENGER, E.L., THOMA, G.E., TOMPKINS, E.A.: Incidence of leukemia following treatment of hyperthyroidism. J. Amer. med. Ass. **205**, 147 (1968).
SCHNEIDER, C.: Behandlung der Thyreotoxikose mit 131-Radiojod. Strahlentherapie **127**, 65 (1965).
SCHULTZ, A.L., ZIEVE, L.: Alterations in thyroid I^{131} uptake, basal metabolic rate and serum cholesterol following treatment of hyperthyroidism with radioactive iodine. Amer. J. Med. **20**, 30 (1956).
SEGAL, R.L., SILVER, S., YOHALEM, S.B., FEITELBERG, S.: Myxedema following radioactive iodine therapy of hyperthyroidism. Amer. J. Med. **31**, 354 (1961).
SHELINE, G.E., LINDSAY, S., BELL, H.G.: Occurrence of thyroid nodules in children following therapy with radioiodine for hyperthyroidism. J. clin. Endocr. **19**, 127 (1959).
— — MCCORMACK, D.B.: Thyroid nodules occurring late after treatment of thyrotoxicosis with radioiodine. J. clin. Endocr. **22**, 8 (1962).
SINCLAIR, W.K., ABBATT, Z., FARRAN, H.E.A., HARRIS, E.B., LAMERTON, L.F.: A quantitative autoradiographic study of radioiodine distribution and dosage in human thyroid glands. Brit. J. Radiol. **29**, 36 (1956).
SMITH, R.N., WILSON, G.M.: Clinical trial of different doses of ^{131}I in the treatment of thyrotoxicosis. Brit. med. J. **1967 I**, 129.
STANBURY, J.B., GROOT, L.J. DE: Problem of hypothyroidism after I^{131} therapy of hyperthyroidism. New Engl. J. Med. **271**, 195 (1964).
UTHGENANNT, H., MÜLLER, W., WEINREICH, J.: Über Häufigkeit und klinische Bedeutung von Autoantikörpern bei Schilddrüsenerkrankungen insbesondere bei der Hyperthyreose. Dtsch. med. Wschr. **91**, 437 (1965).
VIHERKOSKI, M., LAMBERG, B.A., HERNBERG, C.A., NIEMI, E.: Treatment of toxic nodular and diffuse goiter with radioactive iodine. Acta endocr. (Kbh.) **64**, 159 (1970).
WERNER, S.C., JOHNSON, P.M., GOODWIN, P.N., WIENER, J.D., LINDEBOOM, G.A.: Long-term results with iodine-125 treatment for toxic diffuse goiter. Lancet **1970 II**, 681.

Ancillary Treatment of Graves' Disease: Antiadrenergic Agents, Sedatives

BECKER, F.O., SCHWARTZ, T.B.: Sympathetic blockade in hyperthyroidism. Arch. intern. Med. **129**, 967 (1972).
CLARK, F., HALL, R., ORMSTON, B.: Diazepam and tests of thyroid function. Brit. med. J. **1971 I**, 585.
CULLHED, I., PARROW, A.: Acute hemodynamic changes following beta-adrenergic blockade in hyperthyroidism. Acta med. scand. **184**, 235 (1968).
GROSSMAN, W., ROBIN, N.I., JOHNSON, L.W., BROOKS, H., SELENKOW, H.A., DEXTER, L.: Effects of beta blockade on the peripheral manifestations of thyrotoxicosis. Ann. intern. Med. **74**, 875 (1971).
HADDEN, D.R., BELL, T.K., MCDEVITT, D.G., SHANKS, R.G., MONTGOMERY, D.A.D.: Propranolol and the utilization of radioiodine by the human thyroid gland. Acta endocr. (Kbh.) **61**, 393 (1969).
HOWITT, G., ROWLANDS, D.I.: Beta-sympathetic blockade in hyperthyroidism. Lancet **1966 I**, 628.
MCLARTY, D.G., BROWNLIE, B.E.W., ALEXANDER, W.D., PAPAPETROU, P.D., HORTON, P.: Remission of thyrotoxicosis during treatment with propranolol. Brit. med. J. **1973 II**, 332.
SHANKS, R.G., HADDEN, D.R., LOWE, D.C., MCDEVITT, D.G.: Controlled trial of propranolol in thyrotoxicosis. Lancet **1969 I**, 993.
STOUT, B.D., WIENER, L., COX, J.W.: Combined alpha- and beta-sympathetic blockade in hyperthyroidism. Ann. intern. Med. **70**, 963 (1969).
TURNER, P., GRANVILLE-GROSSMAN, K.L., SMART, J.V.: Effect of adrenergic receptor blockade on the tachycardia and axiety state of thyrotoxicosis. Lancet **1965 II**, 1316.
WALDSTEIN, S.S., WEST, G.H., LEE, W.Y., BRONSKY, D.: Guanethidine in hyperthyroidism. J. Amer. med. Ass. **189**, 609 (1964).
WERNER, S.C., PLATMAN, S.R.: Remission of hyperthyroidism and altered pattern of serum-thyroxine binding induced by prednisone. Lancet **1965 II**, 751.
WILSON, W.R., THEILEN, E.O., FLETCHER, F.W.: Pharmacodynamic effects of beta-adrenergic receptor blockade in patients with hyperthyroidism. J. clin. Invest. **43**, 1698 (1964).

Thyroid Crisis

ASHKAR, F.S., KATIMS, R.B., SMOAK, W.M., GILSON, A.J.: Blood exchange and plasmapheresis for thyroid storm. J. Amer. med. Ass. **214**, 1275 (1970).
BUCKLE, R.M.: Treatment of thyroid crisis by beta-adrenergic blockade. Acta endocr. (Kbh.) **57**, 168 (1968).
DILLON, P.T., BABE, J., MELONI, C.R., CANARY, J.J.: Reserpine in thyrotoxic crisis. New Engl. J. Med. **283**, 1020 (1970).
HERRMANN, J., BEISENHERZ, W., GILLICH, K.H,. JESTER, H., KLUGE, R., NISSEN, P., KRÜSKEMPER, H.L.: Peritonealdialyse bei thyreotoxischer Krise. Dtsch. med. Wschr. **50**, 2615 (1969).
HOFFENBERG, R., LOUW, J.H., VOSS, T.J.: Thyroidectomy under hypothermia in a pregnant patient with thyroid crisis. Lancet **1961 II**, 687.
INGBAR, S.H.: Management of emergencies: Thyrotoxic storm. New Engl. J. Med. **274**, 1252 (1966).
LAHEY, F.H.: The crisis of exophthalmic goiter. New Engl. J. Med. **199**, 255 (1928).
LAMBERG, B.A.: The medical thyroid crisis. Acta med. scand. **164**, 479 (1959).
LOGOTHETIS, J., WARNER, J.: Acute thyrotoxic "encephalomyopathy" associated with low serum potassium. Amer. J. Med. **32**, 631 (1962).
MCARTHUR, J.W., RAWSON, R.W., MEANS, J.H., COPE, O.: Thyrotoxic crisis. J. Amer. med. Ass. **134**, 868 (1947).
PIETRAS, R.J., REAL, M.A., POTICHA, G.S., BRONSKY, D., WALDSTEIN, S.S.: Cardio-vascular response in hyperthyroidism. The influence of adrenergic receptor blockade. Arch. intern. Med. **129**, 426 (1972).
ROIZEN, M., BECKER, C.E.: Thyroid storm. Calif. Med. **115**, 5 (1971).
THOMAS, F.B., MAZZAFERRI, E.L., SKILLMAN, T.G.: Apathetic thyrotoxicosis: a distinctive clinical and laboratory entity. Ann. intern. Med. **72**, 679 (1970).
WAHLBERG, P., LAMBERG, B.A.: Iodine metabolism in a case of thyrotoxic crisis. J. clin. Endocr. **23**, 397 (1963).
WALDSTEIN, S.S., SLODKI, S.J., KAGANIE, G.I., BRONSKY, D.: A clinical study of thyroid storm. Ann. intern. Med. **52**, 626 (1960).
WIENER, L., STOUT, B.D., COX, J.W.: Influence of beta-sympathetic blockade (propranolol) on the hemodynamics of hyperthyroidism. Amer. J. Med. **46**, 227 (1969).

Apathetic Thyrotoxicosis

Editorial: Apathetic thyrotoxicosis. Lancet **1970 II**, 809.

RONNOV-JESSEN, V., KIRKEGARD, C.: Hyperthyroidism—A disease of old age? Brit. med. J. **1973 I**, 41.

THOMAS, F. B., MAZZAFERRI, E. L., SKILLMAN, T. G.: Apathetic thyrotoxicosis: A distinctive clinical and laboratory entity. Ann. intern. Med. **72**, 679 (1970).

Graves' Disease in Pregnancy

BURROW, G. N.: The thyroid gland in pregnancy. Philadelphia: W. B. Saunders Co. 1972.

Editorial: Thyrotoxicosis during pregnancy. New Engl. J. Med. **273**, 661 (1965).

ELPHINSTONE, N.: Thiouracil in pregnancy. Its effect on the foetus. Lancet **1953 I**, 1281.

FREEDBERG, I. M., HAMOLSKY, M. W., FREEDBERG, A. S.: The thyroid gland in pregnancy. New Engl. J. Med. **256**, 505, 551 (1957).

GREENBERG, A. H., CZERNICHOW, P., REBA, R. C., TYSON, J., BLIZZARD, R. M.: Observations on the maturation of thyroid function in early fetal life. J. clin. Invest. **49**, 1790 (1970).

HAWE, PH., FRANCIS, H. H.: Pregnancy and thyrotoxicosis. Brit. med. J. **1962 II**, 817.

HERBST, A. L., SELENKOW, H. A.: Hyperthyroidism during pregnancy. New Engl. J. Med. **273**, 627 (1965).

STEIN, R. B., NICOLOFF, J. T.: Thyroid function in pregnancy. Postgrad. Med. **53**, 72 (1973).

ZANINOVICH, A. A.: Effects of oestrogens on thyroxine turnover in hyperthyroidism. Acta endocr. (Kbh.) **71**, 491 (1972).

Neonatal and Childhood Thyrotoxicosis

GAUTIER, E., JUILLARD, E., LEMARCHAND-BÉRAUD, T.: "Long acting thyroid stimulator" et hormone thyréotrope dans la thyréotoxicose du nouveau-né. Schweiz. med. Wschr. **98**, 8 (1968).

HAYLES, A. B.: Problems of childhood Graves' disease. Mayo Clin. Proc. **47**, 850–853 (1972).

— CHAVES-CARBALLO, E., MCCONAHEY, W. M.: Treatment of hyperthyroidism (Graves' disease) in children. Proc. Mayo Clinic **42**, 218 (1967).

JAVETT, S. N., SENIOR, B., BRAUDO, J. L., HEYMANN, S.: Neonatal thyrotoxicosis. Pediatrics **24**, 65 (1959).

KOGUT, M. D., KAPLAN, S. A., COLLIPP, P. J., TIAMSIC, T., BOYLE, D.: Treatment of hyperthyroidism in children. Analysis of forty-five patients. New Engl. J. Med. **272**, 217 (1965).

Leading article: Thyrotoxicosis in children. Brit. med. J. **1965 II**, 1139.

Leading article: Placental transmission of autoimmune diseases. Brit. med. J. **1966 I**, 1554.

LEWIS, I. C., MACGREGOR, A. G.: Congenital hyperthyroidism. Lancet **1957 I**, 14.

MÄENPÄÄ, J., HIEKKALA, H., LAMBERG, B. A.: Childhood hyperthyroidism. Report on 39 cases. Acta endocr. (Kbh.) **51**, 321 (1966).

MCKENZIE, J. M.: Neonatal Graves' disease. J. clin. Endocr. **24**, 660 (1964).

ROSENBERG, D., GRAND, M. J. H., SILBERT, D.: Neonatal hyperthyroidism. New Engl. J. Med. **268**, 292 (1963).

SAXENA, K. M.: Thyrotoxicosis in children. A discussion of present methods of treatment. Clin. Pediat. (Phila.) **4**, 325 (1965).

SCHWARZ, G.: Ein Fall von diaplacentar übertragenem Exophthalmus. Acta endocr. (Kbh.) **30**, 616 (1959).

SUNSHINE, P., KUSUMOTO, H., KRISS, J. P.: Survival time of circulating long-acting thyroid stimulator in neonatal thyrotoxicosis. Pediatrics **36**, 869 (1965).

T_3-Thyrotoxicosis

Editorial: Triiodothyronine. Lancet **1971 I**, 898.

Editorial: Triiodothyronine thyrotoxicosis. J. Amer. med. Ass. **218**, 438 (1971).

HOLLANDER, C. S., MITSUMA, T., NIHEI, N., SHENKMAN, L., BURDAY, S. Z., BLUM, M.: Clinical and laboratory observations in cases of triiodothyronine toxicosis confirmed by radioimmunoassay. Lancet **1972 I**, 609.

— — SHENKMAN, L., STEVENSON, C., PINEDA, G., SILVA, E.: T_3 toxicosis in an iodine-deficient area. Lancet **1972 II**, 1276.

— SHENKMAN, L., MITSUMA, T., BLUM, M., KASTIN, A. J., ANDERSON, D. G.: Hypertriiodothyroninemia as a premonitory manifestation of thyrotoxicosis. Lancet **1971 II**, 731.

IVY, H. K., WAHNER, H. W., GORMAN, C. A.: Triiodothyronine (T_3) toxicosis. Arch. intern. Med. **128**, 529 (1971).

MITSUMA, T., OWENS, R., SHENKMAN, L., REITER, E., HOLLANDER, C. S.: T_3-thyrotoxicosis in childhood. J. Pediat. **81**, 982 (1972).

STERLING, K., BELLABARBA, D., NEWMAN, E. S., BRENNER, M. A.: Determination of triiodothyronine concentration in human serum. J. clin. Invest. **48**, 1150 (1969).

— REFETOFF, S., SELENKOW, M. A.: T_3-thyrotoxicosis. J. Amer. med. Ass. **213**, 571 (1970).

SUNG, L. C., CAVALIERI, R. R.: T_3 thyrotoxicosis due to metastatic thyroid carcinoma. J. clin. Endocr. **36**, 215 (1973).

Endocrine Ophthalmopathy

ADAMS, D. D., PURVES, H. D.: The role of thyrotropin in hyperthyroidism and exophthalmos. Metabolism **6**, 26 (1957).

ARANOW, H., DAY, R. M.: Management of thyrotoxicosis in patients with ophthalmopathy: Antithyroid regimen determined primarily by ocular manifestations. J. clin. Endocr. **25**, 1 (1965).

BAUER, R. K., CATZ, B.: Radioactive iodine therapy for progressive malignant exophthalmos. Acta endocr. (Kbh.) **51**, 15 (1966).

BERMAN, B.: Voluntary propulsion of the eyeballs. The double Whammy syndrome. Arch. intern. Med. **117**, 648 (1966).

BRUNISH, R., HAYASHI, K., HAYASHI, J.: Purification and property of exophthalmos-producing substance. Arch. Biochem. **98**, 135 (1962).

BURROW, G. N., MITCHELL, M. S., HOWARD, R. O., MORROW, L. B.: Immunosuppressive therapy for the eye changes of Graves' disease. J. clin. Endocr. **31**, 307 (1970).

CATZ, B., PERZIK, S. L.: Subtotal vs. total surgical ablation of the thyroid, malignant exophthalmos and its relation to remnant thyroid. In: Current topics in thyroid research. New York-London: Academic Press 1965.

CONDLIFE, P. G.: Chemistry of exophthalmos-producing substance. In: WERNER, S. C., Thyrotropin. Springfield: Thomas 1963.

COLEMAN, D. J., JACK, R. L., FRANZEN, L. A., WERNER, S. C.: High resolution B-scan ultrasonography of the orbit. V. Eye changes of Graves' disease. Arch. Ophthal. **88**, 465 (1972).

CROMBIE, A., LAWSON, A. A. H.: Long-term trial of local guanethidine in treatment of eye signs of thyroid dysfunction and idiopathic lid retraction. Brit. med. J. **1967 IV**, 592.

DESANTO, L. W.: Surgical palliation of ophthalmopathy of Graves' disease. Transantral approach. Mayo Clin. Proc. **47**, 989–992 (1972).

DONALDSON, S. S., BAGSHAW, M. S., KRISS, J. P.: Supervoltage orbital radiotherapy for Graves' ophthalmopathy. J. clin. Endocr. **37**, 276 (1973).

Editorial: Endocrine exophthalmos. Brit. med. J. **1972 III**, 68.

ESSLEN, E., PAPST, W.: Die Bedeutung der Elektromyographie für die Analyse von Motilitätsstörungen der Augen. Basel: Karger 1961.

FURTH, E. D., BECKER, D., RAY, B. S., KANE, J. W.: Appearance of unilateral infiltrative exophthalmos of Graves' disease after the successful treatment of the same process in the contralateral eye by apparently total surgical hypophysectomy. J. clin. Endocr. **22**, 518 (1962).

GARBER, M. I.: Methylprednisolone in the treatment of exophthalmos. Lancet **1966 I**, 958.

GREEN, D. E., SNYDER, N. J., SOLOMON, D. H.: Gluco-corticoid-induced disappearance of the long-acting thyroid stimulator in Graves' ophthalmopathy. J. clin. Invest. **42**, 939 (1963).

GREIG, W. R., ABOUL-KHAIR, S. A., MOHAMED, S. D., CROOKS, J.: Effect of treatment of thyrotoxicosis on exophthalmos. Brit. med. J. **1965 II**, 509.

HADDAD, H. M.: Endocrine exophthalmus. J. Amer. med. Ass. **199**, 559 (1967).

HALL, R., DONIACH, D., KIRKHAM, K.. EL KABIR, D.: Ophthalmic Graves' disease. Lancet **1970 I**, 375.

HAMBURGER, J. I., SUGAR, H. S.: What the internist should know about the ophthalmopathy of Graves' disease. Arch. intern. Med. **129**, 131 (1972).

HAMILTON, R. D., MAYBERRY, W. E., MCCONAHEY, W. M., HANSON, K. C.: Ophthalmopathy of Graves' disease: Comparison between surgical and radioiodide therapy. Mayo Clin. Proc. **42**, 812 (1967).

HORST, W., SAUTTER, H., ULLERICH, K.: Radiojoddiagnostik und Strahlentherapie der endokrinen Ophthalmopathie. Dtsch. med. Wschr. **85**, 730, 794 (1960).

HORSTER, F. A., SCHLEUSENER, H., SCHIMMELPFENNING, K.: Die Bedeutung von thyreotropem Hormon (TSH), long acting thyroid stimulator (LATS) und exophthalmusproduzierendem Faktor (EPF) bei verschiedenen Formen der Schilddrüsenüberfunktion. Dtsch. med. Wschr. **92**, 673 (1967).

HUBER, A., ESSLEN, E.: Diagnostic des Myopathies oculaires à l'aide de l'électromyographie. Bull. neurol. Soc. franç. Ophthal. **80**, 460 (1967).

IGERSHEIMER, J.: Visual changes in progressive exophthalmos. Arch. Ophthal. **53**, 94 (1955).

IVY, H. K.: Medical approach to ophthalmopathy of Graves' disease. Mayo Clin. Proc. **47**, 980–985 (1972).

JALLUT, O., GALLETTI, P. M.: L'exophtalmie maligne (syndrome thyréohypophysaire) Présentation de 28 cas. Schweiz. med. Wschr. **90**, 639 (1960).

KINDEREN, P. J. DER: EPS, LATS and Exophthalmos. In: IRVINE, W. J., Thyrotoxicosis. Edinburgh-London: Livingstone 1967.

— HOUTSTRA-LANZ, M., SCHWARZ, F.: Exophthalmos-producing substance in human serum. J. clin. Endocr. **20**, 712 (1960).

KOHN, L. D., WINAND, R. J.: Relationship of thyrotropin to exophthalmus-producing substance. J. biol. Chem. **246**, 6570 (1971).

KOUTRAS, D. A., ALEXANDER, W. D., BUCHANAN, W. W., MCG. HARDEN, R.: Effect of thyroxine on exophthalmos in thyrotoxicosis. Brit. med. J. **1965 I**, 493.

— BOUZAS, A. G., LIVIDAS, D. P., VAGENAKIS, A. P., KOUKOULOMMATI, A. S.: Comparative drug treatment of endocrine exophthalmos. Europ. J. clin. Pharmacol. **3**, 32 (1970).

KRAYENBÜHL, H.: The value of orbital angiography for diagnosis of unilateral exophthalmos. J. Neurosurg. **4**, 289 (1962).

KRISS, J. P., ROSENBLUM, A. L., HOLDERNESS, M., SHARP, G., UTIGER, R.: Studies on the pathogenesis of the ophthalmopathy of Graves' disease. J. clin. Endocr. **27**, 582 (1967).

LAMBERG, B. A.: The thyro-hypophysical syndrome. I. The primary reaction of the hypophysical eye signs (including exophthalmos) to the treatment of thyrotoxicosis. Acta med. scand. **148**, 225 (1954).

— The thyro-hypophysial syndrome. II. Roentgen irradiation of the pituitary region in the treatment of the hypophysial eye signs (including exophthalmos) after thyroidectomy. Acta med. scand. **156**, 361 (1957).

— HERNBERG, C. A.: The thyro-hypophysial syndrome. III. Pituitary roentgen irradiation in the treatment of the hypophysial eye signs (including exophthalmos) during treatment of thyrotoxicosis with thyrostatic drugs. Acta med. scand. **156**, 378 (1957).

— The thyro-hypophysial syndrome. IV. Hypophysial eye signs (including exophthalmos) without thyrotoxicosis (solitary thyro-hypophysial syndrome) and their treatment by roentgen irradiation of the pituitary region. Acta med. scand. **156**, 392 (1957).

LIPMAN, L. M., GREEN, D. E., SNYDER, N. J., NELSON, J. C., SOLOMON, D. H.: Relationship of long-acting thyroid stimulator to the clinical features and course of Graves' disease. Amer. J. Med. **43**, 486 (1967).

MAHIEU, P., WINAND, R.: Demonstration of delayed hypersensitivity to retrobulbar and thyroid tissue in human exophthalmos. J. clin. Endocr. **34**, 1090 (1972).

MCGILL, D. A., ASPER, S. A., JR.: Endocrine exophthalmos: a review and a report on antibody studies. New Engl. J. Med. **267**, 133/188 (1962).

MCKENZIE, J. M., MCCULLAGH, E. P.: Observations against a causal relationship between the long-acting thyroid stimulator and ophthalmopathy of Graves' disease. J. clin. Endocr. **28**, 1177 (1968).

MORGAN, D. C., MASON, A. S.: Exophthalmos in Cushing's syndrome. Brit. med. J. **1958 II**, 481.

NAFFZIGER, H. C., JONES, O. W., JR.: The surgical treatment of progressive exophthalmos following thyroidectomy. J. Amer. med. Ass. **99**, 638 (1932).

OGURA, J. H., WALSH, I. E.: The transantral orbital decompression operation for progressive exophthalmos. Laryngoscope (St. Louis) **72**, 1078 (1962).

OSSOINIG, K.: Echographie der Orbita. Methode der Wahl für die Diagnostik des einseitigen Exophthalmus. Schweiz. med. Wschr. **99**, 1034 (1969).

PEQUEGNAT, E. P.: Large doses of radioiodide in Graves' disease: Effect on ophthalmopathy and long-acting thyroid stimulator. Mayo Clin. Proc. **42**, 802 (1967).

PLIMSTONE, B. L., HOFFENBERG, R., BLACK, E.: Parallel assays of thyrotrophin, long-acting thyroid stimulator and exophthalmos-producing substance in endocrine exophthalmos and pretibial myxedema. J. clin. Endocr. **24**, 976 (1964).

RILEY, F. C.: Surgical management of ophthalmopathy in Graves' disease. Transfrontal orbital decompression. Mayo Clin. Proc. **47**, 986–988 (1972).

SCHWARZ, F., KINDEREN, P. J. DER, HOUTSTRA-LANZ, M.: The correlation between endocrine exophthalmos and exophthalmos producing substance (EPS). Acta endocr. (Kbh.) **51**, 359 (1966).

SEGAL, R. L., ROSENBLATT, S., ELIASOPH, I.: Endocrine exophthalmos during lithium therapy of manic depressive disease. New Engl. J. Med. **289**, 136 (1973).

SMELSER, G. K.: Experimental studies on exophthalmus. Amer. J. Ophthal. **54**, 929 (1962).

SNYDER, J. N., GREEN, D. E., SOLOMON, D. H.: Glucocorticoid-induced disappearance of long-acting thyroid stimulator in the ophthalmopathy of Graves' disease. J. clin. Endocr. **24**, 1129 (1964).

SUMMERSKILL, W. H. J., MOLNAR, G. D.: Eye signs in hepatic cirrhosis. New Engl. J. Med. **266**, 1244 (1962).

VOLPÉ, R., DESBARATS-SCHONBAUM, M. L., SCHONBAUM, E., ROW, V. V., EZRIN, C.: The effect of radioablation of the thyroid gland in Graves' disease with high levels of long-acting thyroid stimulator (LATS). Amer. J. Med. **46**, 217 (1969).

WEGELIUS, O., ASBOE-HANSEN, G., LAMBERG, B. A.: Retrobulbar connective tissue changes in malignant exophthalmos. Acta endocr. (Kbh.) **25**, 452 (1957).

WERNER, S. C.: The severe eye changes of Graves' disease. J. Amer. med. Ass. **177**, 551 (1961).
— Prednisone in emergency treatement of malignant exophthalmos. Lancet **1966 I**, 1004.
— Immunosuppression in the management of the active severe eye changes of Graves' disease. In: IRVINE, W. J., Thyrotoxicosis. Edinburgh-London: Livingstone 1967a.
— Classification of the eye changes of Graves' disease. J. clin. Endocr. **29**, 782 (1969).
— The eye changes of Graves' disease. Overview. Mayo Clin. Proc. **47**, 969 (1972).
— FEIND, C. R., AIDA, M.: Graves' disease and total thyroidectomy. Progression of severe eye changes and decrease in serum long-acting thyroid stimulator after operation. New Engl. J. med. **276**, 132 (1967b).
WINAND, R. J., KOHN, L. D.: The binding of (^{3}H) thyrotropin and ^{3}H-labeld exophthalmogenic factor by plasma membranes of retroorbital tissue. Proc. nat. Acad. Sci. (Wash.) **69**, 1711 (1972).
WYSE, E. P., MCCONAHEY, W. M., WOOLNER, L. B., SCHOLZ, D. A., KEARNS, TH. P.: Ophthalmopathy without hyperthyroidism in patients with histologic Hashimoto's thyroiditis. J. clin. Endocr. **28**, 1623 (1968).
YASARGIL, M. G.: Die Röntgendiagnostik des Exophthalmus unilateralis. Basel-New York: Karger 1957.
ZIZMOR, J., FASANO, C. V., SMITH, B., RABETT, W.: Roentgenographic diagnosis of unilateral exophthalmos. J. Amer. med. Ass. **197**, 343 (1966).

Toxic Nodular Goiter (Toxic Adenoma)

BAY, V.: Das toxische Adenom der Schilddrüse. Ergebn. Chir. Orthop. **47**, 132 (1965).
BECKER, F. O., ECONOMO, P. G., SCHWARTZ, T. B.: Occurrence of carcinoma in "hot" thyroid nodules: report of two cases. Ann. intern. Med. **58**, 877 (1963).
BURKE, C., SZABO, M.: Dissociation of in vivo and in vitro "autonomy" in hyperfunctioning thyroid nodules. J. clin. Endocr. **35**, 199 (1972).
COPE, O., RAWSON, R. W., MCARTHUR, J. W.: The hyperfunctioning single adenoma of the thyroid. Surg. Gynec. Obstet. **84**, 415 (1947).
ENGELBRECHT, W.: Zürcher Ergebnisse der Radioresektion bei 698 Patienten mit Morbus Basedow und der Radioelimination bei 404 Patienten mit toxischem Adenom — 1102 Patienten mit Hyperthyreose. Thesis Zürich, 1970.
ERMANS, A. M., CAMUS, M.: Evidence of a regulation of the thyroid activity by the level of dietary iodine in some thyroid disorders. In: Further Adv. Thyroid Res. (K. FELLINGER, R. HÖFER, eds.), p. 85. Vienna: Wiener Med. Akademie 1971.
FELLINGER, K., HÖFER, R., EGERT, H., VETTER, H.: Clinical and laboratory observations in patients with hyperactive thyroid nodules. Advances in thyroid research, p. 347. London: Pergamon Press 1961.
FERRIMAN, D., HENNEBRY, T. M., TASSOPOULOS, C. N.: True thyroid adenoma. Quart. J. Med. (N. S.) **41**, 127 (1972).
FUCHSIG, P.: Die Struma: weder ein internistisches noch chirurgisches, sondern ein ärztliches Problem. Chirurg **39**, 158 (1968).
GARNIER, H., REYNIER, J., SAVOIE, J. C., CALMETTES, C., MEILLÈRE, D., CORDIER, G.: L'adénome toxique du corps thyroïde. A propos de 100 cas opérés. Ann. Chir. **18**, 264 (1964).
GILBERT-DREYFUS, GALI, P.: Les critères de guérison des adenomes toxiques traités par l'iode radioactif. Rév. franç. Endocr. clin. **4**, 27 (1963).
GREENE, R., FARRAN, H. E. A.: On single "hot" nodules of the thyroid gland. J. Endocr. **33**, 537 (1965).
GUINET, P., TOURNIAIRE, J., GUILLAUD, M., BRIERE, J., DALMAIS, J., CHALENDAR, D.: Adénome toxique et cancer thyroïdien. Ann. Endocr. (Paris) **32**, 513 (1971).
HALL, R.: Hyperthyroidism. Pathogenesis and diagnosis. Brit. med. J. **1970 I**, 743.
HORST, W., RÖSLER, H., SCHNEIDER, C., HEINZEL, F., CONRAD, B.: Differentialdiagnostik, Therapie und Pathophysiologie des toxischen Adenoms. In: Radio-Isotope in der Endokrinologie. Stuttgart: F. K. Schattauer 1965.
— — — LABHART, A.: 306 cases of toxic adenoma: clinical aspects, findings in radiochromatography and histology, radioiodine diagnostics, results of I^{131} and surgical therapy. J. nucl. Med. **8**, 515 (1967).
— SCHNEIDER, C., THIEMANN, K, J.: Ergebnisse der Radiopapierchromatographie, Szintigraphie und Radioelimination bei 58 Fällen von toxischem Adenom der Schilddrüse. Verh. Dtsch. Ges. Inn. Med. 66. Kongr. p. 356, 1960.
KARLBERG, B. E.: Thyroid nodule autonomy: Its demonstration by the thyrotrophin releasing hormone (TRH) stimulation test. Acta endocr. (Kbh.) **73**, 689 (1973).
KRAMPF, K., AKOVBIANTZ, A., RÖSLER, R.: Diagnose und Therapie des toxischen Schilddrüsenadenoms. Helv. chir. Acta **34**, 132 (1967).
LARSEN, P. R., YAMASHITA, K., DEKKER, A., FIELD, J. B.: Biochemical observations in functioning human thyroid adenomas. J. clin. Endocr. **36**, 1009 (1973).
MOLNAR, G. D., CHILDS, D. S., WOOLNER, L. B.: Histologic evidence of malignancy in a thyroid gland bearing a "hot" nodule. J. clin. Endocr. **18**, 1132 (1958).
— WILBER, R. D., LEE, R. E., WOOLNER, L. B., KEATING, F. R., JR.: On the hyperfunctioning solitary thyroid nodule. Proc. Mayo Clinic **40**, 665 (1965).
PLUMMER, H. S.: The clinical and pathological relationship of simple and exophthalmic goiter. Trans. Ass. Amer. Physns **28**, 587 (1913).
— The clinical and pathologic relationships of hyperplastic and nonhyperplastic goiter. J. Amer. med. Ass. **61**, 650 (1913).
POHL, G., GALVAN, G., STEINER, H., SALIS-SAMADEN, R.: Das autonome Adenom der Schilddrüse im Struma-Endemie-Gebiet. Dtsch. med. Wschr. **98**, 189 (1973).
SAVOIE, J. C.: Etude clinique et biologique de quarante-trois cas d'adénome toxique thyroïdien. Rev. franç. Étud. clin. biol. **6**, 263 (1961).
SHELINE, G. E., MCCORMACK, K.: Solitary hyperfunctioning thyroid nodules. J. clin. Endocr. **20**, 1401 (1960).
SHIMAOKA, K.: Toxic adenoma of the thyroid with triiodothyronine as the principal circulating thyroid hormone. Acta endocr. (Kbh.) **43**, 285 (1963).
SILVERSTEIN, G. E., BURKE, G., COGAN, R.: The natural history of the autonomous hyperfunctioning thyroid nodule. Ann. intern. Med. **67**, 539 (1967).
SKILLERN, P. G., MCCULLAGH, E. P., CLAMEN, M.: Radioiodine in diagnosis and therapy of hyperthyroidism caused by hyperfunctioning thyroid adenoma. Arch. intern. Med. **110**, 888 (1962).
STERLING, K., REFETOFF, S., SELENKOW, H. H. A.: T_3-Thyrotoxicosis. J. Amer. med. Ass. **213**, 571 (1970).
VIGIER, R., VON: Probleme der Hyperthyreosediagnostik. Helv. med. Acta **31**, 191 (1964).
WERNER, S. C.: Toxic goiter. In: The thyroid, 3rd ed. (S. C. WERNER and S. H. INGBAR, eds.), p. 491. New York: Harper & Row 1972.
ZUKSCHWERDT, L., BAY, V.: Die gezielte Operationstechnik im Nichtendemiegebiet. Wien. med. Wschr. **113**, 823 (1963).

Factitious Thyrotoxicosis, Struma ovarii

BROWN, W. W., RETTY, K. R., ROSENFELD, P. S.: Hyperthyroidism due to struma ovarii: demonstration by radioiodine scan. Acta endocr. (Kbh.) **73**, 266 (1973).
HARVEY, R. F.: Thyroxine "addicts". Brit. med. J. **1973 II**, 35.

KIRKEBY, K.: Fatty acid composition of serum lipids in hyper- and hypothyroidism. Acta endocr. (Kbh.) **71**, 73 (1972).

ROSE, E., SANDERS, T. P., WEBB, W. L., HINES, R. C.: Occult factitial thyrotoxicosis. Ann. intern. Med. **71**, 309 (1969).

Iodine-Induced Thyrotoxicosis

ADAMS, D. D., KENNEDY, T. H., CHOUFOER, J. C., QUERIDO, A.: Endemic goiter in Western New Guinea. III. Thyroid stimulating activity of serum from severely iodine-deficient people. J. clin. Endocr. **28**, 685 (1968).

CONNOLLY, R. J., VIDOR, G. I., STEWART, J. C.: Increase in thyrotoxicosis in endemic goiter area after iodation of bread. Lancet **1970 I**, 500.

ERMANS, A. M., CAMUS, M.: Evidence of a regulation of the thyroid activity by the level of dietary iodine in some thyroid disorders. In: Further Adv. Thyroid Res. (K. FELLINGER, R. HÖFER, eds.), p. 85. Vienna: Wiener Med. Akademie 1971.

KOCHER, TH.: Über Jodbasedow. Langenbecks Arch. klin. Chir. **92**, 1166 (1910).

LAROCHE, G., HIRSCH, M.: Les troubles thyroïdiens d'origine iodée: goitre, iode-myxoedème, iode-Basedow. Presse méd. **69**, 2219 (1960).

MATOVINOVIC, J., RAMALINGASWAMI, V.: Therapy and prophylaxis of endemic goiter. In: Endemic goiter. Geneva: WHO 1960.

QUERVAIN, F. DE: Zur Kropfprophylaxe durch Jodkochsalz. Schweiz. med. Wschr. **59**, 1099 (1929).

RILLIET, F.: Sur le iodisme constitutionel. Bull. Acad. imp. Med. **25**, 382, 479 (1859/60).

ROCH, M.: Le corps thyroïde et le métabolisme de l'iode. In Glandes endocrines et vitamines. Genève: Les presses accadémiques 1943.

STANBURY, J. B., BROWNELL, G. L., RIGGS, D. S., PERINETTI, H., HOIZ, J., CASTILLO, E. B. DEL: Endemic goiter, the adaptation of man to iodine deficiency. Cambridge, Mass. USA: Harvard University Press 1954.

VAGENAKIS, A. G., WANG, C., BURGER, A., MALOOF, F., BRAVERMAN, L. E., INGBAR, S. H.: Iodide-induced thyrotoxicosis in Boston. New Engl. J. Med. **287**, 523 (1972).

Hydatidiform Mole and Choriocarcinoma

BURGER, A.: Further studies on a thyroid stimulating factor in crude chorionic gonadotrophin preparations and in urine. Acta endocr. (Kbh.) **55**, 587 (1967).

GALTON, V. A., INGBAR, S. H., JIMENEZ-FONESCA, J., HERSHMAN, J. M.: Alterations in thyroid hormone economy in patients with hydatidiform mole. J. clin. Invest. **50**, 1345 (1971).

HERSHMAN, J. M., HIGGINS, H. P.: Hydatidiform mole—A cause of clinical hyperthyroidism. New Engl. J. Med. **284**, 573 (1971).

— — STARNES, W. R.: Differences between thyroid stimulator in hydatiform mole and human chorionic thyrotropin. Metabolism **19**, 735 (1970).

ODELL, W. D., BATES, R. W., RIOLIN, R. S., LIPSETT, M. B., HERTZ, R.: Increased thyroid function without clinical hyperthyroidism in patients with choriocarcinoma. J. clin. Endocr. **23**, 658 (1963).

STEIGBIGEL, N. H., OPPENHEIM, J. J., FISHMAN, L. M., CARBONE, P. P.: Metastatic embryonal carcinoma of the testis associated with elevated plasma TSH-like activity and hyperthyroidism. New Engl. J. Med. **271**, 345 (1964).

Thyrotoxicosis Due to Thyroid Carcinoma

FEDERMAN, D. D.: Hyperthyroidism due to functioning metastatic carcinoma of the thyroid. Medicine (Baltimore) **43**, 267 (1964).

GHOSE, M. K., GENUTH, S. M., ABELLERA, R. M., FRIEDMAN, S., LIDSKY, I.: Functioning primary thyroid carcinoma and metastases producing hyperthyroidism. J. clin. Endocr. **33**, 639 (1971).

HUNT, W. B., CRISPELL, K. R., MCKEE, J.: Functioning metastatic carcinoma of the thyroid producing clinical hyperthyroidism. Amer. J. Med. **28**, 995 (1960).

STUDER, H., VERAGUTH, P., WYSS, F.: Thyrotoxicosis due to a solitary hepatic metastasis of thyroid carcinoma. J. clin. Endocr. **11**, 1334 (1961).

Thyrotoxicosis Due to TSH Producing Pituitary Tumors

EMERSON, C. H., UTIGER, R. D.: Hyperthyroidism and excessive thyrotropin secretion. New Engl. J. Med. **287**, 328 (1972).

FAGLIA, G., FERRARI, C., NERI, V., BECK-PECCOZ, B., AMBROSI, B., VALENTINI, F.: High plasma thyrotrophin levels in two patients with pituitary tumor. Acta endocr. (Kbh.) **69**, 649 (1972).

HAMILTON, C. R., ADAMS, L. C., MALOOF, F.: Hyperthyroidism due to thyrotropin-producing pituitary chromophobe adenoma. New Engl. J. Med. **283**, 1077 (1970).

— MALOOF, F.: Acromegaly and toxic goiter. J. clin. Endocr. **35**, 659 (1972).

MORNEX, R., TOMMASI, M., CURE, M., FARCOT, J., ORGIAZZI, J., ROUSSET, B.: Hyperthyroïdie associée à un hypopituitarisme au cours de l'évolution d'une tumeur hypophysaire sécrétant T.S.H. Ann. Endocr. (Paris) **33**, 390 (1972).

Endemic Goiter and Cretinism

ADAMS, D. D., KENNEDY, T. H., CHOUFOER, J. C., QUERIDO, A.: Endemic goiter in Western New Guinea. III. Thyroid stimulating activity of serum from severely iodine-deficient people. J. clin. Endocr. **28**, 685 (1968).

ALEXANDER, W. D., KOUTRAS, D. A., CROOKS, J., BUCHANAN, W. W., MACDONALD, E. M., RICHMOND, M. H., WAYNE, E. J.: Quantitative studies of iodine metabolism in thyroid disease. Quart. J. Med. **31**, 281 (1962).

ASTWOOD, E. B., CASSIDY, C. E., AURBACH, G. D.: Treatment of goiter and thyroid nodules with thyroid. J. Amer. med. Ass. **174**, 459 (1960).

BAHNER, F.: Erfahrungen über die Behandlung der Struma mit Schilddrüsenhormonen. In: Testosteron-Schilddrüse. 13. Symp. Dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.

BANSI, H. W.: Zur Prophylaxe und internen Therapie der Adoleszenten- und Erwachsenenstruma. Internist (Berl.) **4**, 322 (1963).

BASTENIE, P. A., ERMANS, A. M., THYS, O., BECKERS, C., SCHRIECK, H. G. VAN DEN, VISSCHER, M. DE: Endemic goiter in the Uele region. III. Endemic cretinism. J. clin. Endocr. **22**, 187 (1962).

BECKERS, CH.: L'hormonogénèse dans les goitres endémiques et sporadiques. Bruxelles: Arscia 1962.

BECKERS, C., VISSCHER, M. DE: Proteolytic activity of thyroid enzymes in endemic goiter. J. clin. Endocr. **22**, 711 (1962).

BEIERWALTES, W. H., DODSON, V. N., WHEELER, A. H.: Thyroid antibodies in the families of cretins. J. clin. Endocr. **19**, 179 (1959).

BERGFELT, G., RISHOLM, L.: Postoperative thyroid hormone therapy in nontoxic goitre. Acta chir. scand. **126**, 531 (1963).

BUTTFIELD, I. H., BLACK, M. L., HOFFMANN, M. J., MASON, E. K., HETZEL, B. S.: Iodised oil injection. Lancet **1965 II**, 767.

— — — — WELLBY, M. L., GOOD, B. F., HETZEL, B. S.: Studies of the control of thyroid function in endemic goiter in Eastern New Guinea. J. clin. Endocr. **26**, 1201 (1966).

— HETZEL, B.S., ODELL, W.D.: Effect of iodized oil on serum TSH determined by immunoassay in endemic goiter subjects. J. clin. Endocr. **28**, 1664 (1968).
CARR, E.A., JR., BEIERSWALTES, W.H., RAMAN, G., DODSON, V.N., TANTAN, J., BETTS, J.S., STAMBAUGH, R.A.: The effect of maternal thyroid function on fetal thyroid function and development. J. clin. Endocr. **19**, 1 (1959).
CASSANO, C., BASCHIERI, L., ANDREANI, D.: Etude de 48 cas de goitre simple avec élévation de la clearance rénale de l'iode. In: Advances in thyroid research (R. PITT-RIVERS). Oxford: Pergamon Press 1961.
CHOUFOER, J.C., RHIJN, M. VAN, KASSENAAR, A.A.H., QUERIDO, A.: Endemic goiter in western New Guinea: Iodine metabolism in goitrous and nongoitrous subjects. J. clin. Endocr. **23**, 1203 (1963).
— — QUERIDO, A.: Endemic goiter in western New Guinea. II. Clinical picture, incidence and pathogenesis of endemic cretinism. J. clin. Endocr. **25**. 385 (1965).
CLEMENTS, F.W.: Health significance of endemic goitre and related conditions. Endemic goitre. Geneva: World Health Organization 1960.
COBLE, Y.D., KOHLER, P.O.: Plasma TSH levels in endemic goiter subjects. J. clin. Endocr. **31**, 320 (1970).
CONOLLY, R.J., VIDOR, G.I., STEWART, J.C.: Increase in thyrotoxicosis in an endemic goitre area after iodination of bread. Lancet **1970I**, 500.
COSTA, A.: Has endemic cretinism any relation to iodine deficiency? J. clin. Endocr. **17**, 801 (1957).
— COITINO, F.: Research on iodine metabolism in endemic goiter in Piedmont. Metabolism **12**, 35 (1963).
— — MORTARA, M., VOGLIAZZO, U.: Endemic cretinism in Piedmont. Panminerva med. **6**, 250 (1964).
— MORTARA, M.: A review of recent studies of goitre in Italy. Bull. Wld. Hlth. Org. **22**, 493 (1960).
— — COTTINO, F., PELLERITO, N., DALL'ACQUA, R.: Recherches sur la fonction de la thyroïde, l'électroencephalographie et la structure du squelette dans le crétinisme endémique. Ann. Endocr. (Paris) **20**, 237 (1959).
DAY, T.K., POWELL-JACKSON, P.R.: Fluoride, water hardness and endemic goiter. Lancet **1972II**, 1135.
DELANGE, F.: Le goitre endémique de l'ile d'Idjwi (Lac Kivu, République du Congo). Données préliminaires. Ann. Endocr. (Paris) **27**, 256 (1966).
— CAMUS, M., ERMANS, A.M.: Circulating thyroid hormones in endemic goiter. J. clin. Endocr. **34**, 891 (1972).
— ERMANS, A.M., VIS, H.L., STANBURY, J.B.: Endemic cretinism in Idjwi island. J. clin. Endocr. **34**, 1059 (1972).
— THILLY, C., ERMANS, A.M.: Iodine deficiency, a permissive condition in the development of endemic goiter. J. clin. Endocr. **28**, 114 (1968).
DIETERLE, D.: Beitrag zur Kasuistik der Vererbungsfrage des endemischen Kretinismus. Untersuchung an blutsverwandten kretinen Eltern und deren Nachkommen. Arch. Klaus-Stift. Vererb.-Forsch. **27**, 69 (1952).
DIMITRIACLOU, A., SUWANIK, R., FRASER, T.R., PEARSON, J.D.: Endemic goiter in Thailand. J. Endocr. **34**, 23 (1966).
DUMONT, J.E., ERMANS, A.M., BASTENIE, P.A.: Thyroid function in a goiter endemic. IV. Hypothyroidism and endemic cretinism. J. clin. Endocr. **23**, 325 (1963).
EAYRS, J.T.: Effects of thyroid hormones on brain differentiation. In: Brain-thyroid relationships, edit. by M.P. CAMERON and M.O'CONNOR. Ciba found. Study group No 18. London: Curchill 1964.
— Thyroid and central nervous development. In: The scientific basis of medicine. London: Athlone 1966.
EGGENBERGER, H.: Kropf und Kretinismus. In: HIRSCH. Handbuch der inneren Sekretion. Leipzig: C. Kabitzsch 1932.
EGLOFF, B.: Bösartige Schilddrüsengeschwülste mit besonderer Berücksichtigung maligner Rezidive primär gutartiger Kröpfe. Schweiz. med. Wschr. **91**, 424 (1961).
EKPECHI, O.L., DIMITRIADOU, A., FRASER, T.R.: Food goitrogens from Nigeria. In: Current topics in thyroid research. Ed. by CASSANO, C., ANDREOLI, M. New York: Academic Press 1965.
ERMANS, A.M., BASTENIE, P.A., GALPERIN, H., BECKERS, C., SCHRIECK, H.G. VAN DEN, VISSCHER, M. DE: Endemic goiter in the Uele region. II. Synthesis and secretion of thyroid hormones. J. clin. Endocr. **21**, 996 (1961).
— DUMONT, J.E., BASTÉNIE, P.A.: Thyroid function in a goiter endemic. I: Impairment of hormone synthesis and secretion in the goitrous gland. J. clin. Endocr. **23**, 539 (1963).
— — — Thyroid function in a goiter endemic: II. Nonhormonal iodine escape from the goitrous gland. J. clin. Endocr. **23**, 550 (1963).
— KINTHAERT, J., CAMUS, M.: Defective intrathyroidal iodine metabolism in nontoxic goiter. Inadequate iodination of thyroglobulin. J. clin. Endocr. **28**, 1307 (1968).
EUGSTER, J.: Zur Erblichkeitsfrage des endemischen Kretinismus. Untersuchungen an 204 Kretinen und deren Blutsverwandten. Arch. Klaus-Stift. Vererb.-Forsch. **12**, 383 (1938).
— Endemic goiter and cretinism. Investigations based on more than 15000 clinical observations. 3rd Internat. goiter conference; transactions. Portland, Ore, American Ass. Goiter 1938.
— Wie sieht die Schilddrüse eines Kretinen zur Zeit der Geburt? Beitr. path. Anat. **100**, 392 (1938).
— Zur Erblichkeitsfrage des endemischen Kretinismus. Fortschr. Erbpath., Rassenhyg. **5**, 156 (1941).
FAGGE, C.H.: On sporadic cretinism, occurring in England. Med.-chir. Trans. **54**, 155 (1871).
FELLENBERG, TH. v.: Kropf und Trinkwasser in der Schweiz. Mitt. Lebensmitt. Hrsg. **24**, 123 (1933).
— Das Vorkommen, der Kreislauf und der Stoffwechsel des Jods. Ergebn. Physiol. **25**, 176 (1926).
FIERRO-BENITEZ, R., RAMIREZ, I., SUAREZ, J.: Effect of iodine correction early in fetal life on intelligence quotient. A preliminary report. In: Human development and the thyroid gland (J.B. STANBURY and R.L. KROC, eds.), p. 239. New York: Plenum Press 1972.
GAITAN, E., ISLAND, D.P., LIDDLE, G.W.: Identification of goitrogenic substances in water. Clin. Res. **17**, 65 (1969).
GHALIOUG NUI, P.: Thyroid enlargement in Africa with reference to the Nile basin. Nat. inform document. center. Nidoc, Cairo, 1965.
GOLDSCHMIDT, V.M.: Geochemistry. (ed.). A. MUIR (ed.) Oxford: Clarendon Press 1954.
GREENBERG, A.H., CZERNICHOW, P., REBA, R.C., TYSON, J., BLIZZARD, R.M.: Observations on the maturation of thyroid function in early fetal life. J. clin. Invest. **49**, 1790 (1970).
GREER, M.A.: The natural occurrence of goitrogenic agents. Recent Progr. Hormone Res. **18**. 187 (1962).
GYR, N.: Der Einfluß der Jodsalzprophylaxe auf die Gesamtzahl der Kropfoperationen in der Schweiz im Zeitraum von 1945—1961. Praxis **53**, 1382 (1964).
HUNZIKER, H.: Kropfmessungen am Lebenden. Schweiz. med. Wschr. **50**, 87 (1920).
— Die Prophylaxe der großen Schilddrüse. Bern, Leipzig: Bircher 1924.
JENNY, H., BLOCK, M.A., HORN, R.C., MILLER, J.M.: Recurrence following surgery for benign thyroid nodules. Arch. Surg. **92**, 525 (1966).
KEIDERLING, W., EMRICH, D., HAUSWALDT, CH., HOFFMANN, G.: Ergebnisse der Radiojod-Verkleinerungstherapie euthyreoter Strumen. Dtsch. med. Wschr. **89**, 453 (1964).
KELLY, F.C., SNEDDEN, W.W.: Prevalence and geographical distribution of endemic goitre. In: Endemic goitre, p. 27. Geneva: World Health Organization 1960.
KIČIČ, M., MILUTINOVIC, P., DJORDJEVIC, S., RAMZIN, S.: Endocrinological aspect of an endemic focus of cretinism. In: R. PITT-RIVERS, Advances in thyroid research. London: Pergamon Press 1961.

KILPATRICK, R., MILNE, J.S., RUSHBROOKE, M., WILSON, E.S.B., WILSON, G.M.: A survey of thyroid enlargement in two general practices in Great Britain. Brit. med. J. **1963 I**, 29.

KITCHIN, F.D., EVANS, W.H.: Genetic factors in thyroid diseases. Brit. med. Bull. **16**, 148 (1960).

KLEIN, E.: Strumen im Wachstumsalter. Internist (Berl.) **6**, 30 (1965).

KÖNIG, M.P.: Nutzen und Gefahren der Kropfprophylaxe mit Jod. Internist (Berl.) **4**, 327 (1963).

— Die kongenitale Hypothyreose und der endemische Kretinismus. Berlin-Heidelberg-New York: Springer 1968.

— NEIGER, M.: The pathology of the ear in endemic cretinism. In: Human development and the thyroid gland (J.B. STANBURY and R.L. KROC, eds.), p. 325. New York: Plenum Press 1972.

LAMBERG, B.A., HINTZE, G.: Das Syndrom des butanol-unlöslichen Jodproteins im Serum erwachsener eumetabolischer Kropfpatienten. Dtsch. med. Wschr. **88**, 434 (1963).

— HONKAPOHJA, H., HAIKONEN, M., JUSSILA, R., HINTZE, G., AXELSON, E., CHOUFOER, J.C.: Iodine metabolism in endemic goitre in the east of Finland with a survey of recent data on iodine metabolism in Finland. Acta med. scand. **172**, 237 (1962).

LEMARCHAND-BÉRAUD, T., SCAZZIGA, T., VANNOTTI, A.: Plasma thyrotropin levels in thyroid diseases and effect of treatment. Acta endocr. (Kbh.) **62**, 593 (1969).

LOBO, L.C.G., POMPEN, F., ROSENTHAL, D.: Endemic cretinism in Goiaz, Brazil. J. clin. Endocr. **23**, 407 (1963).

LOGOTHETOPOULOS, J., SCOTT, R.F.: Active iodide transport across the placenta of the guinea-pig, rabbit and rat. J. Physiol. (Lond.) **132**, 365 (1956).

LUCA, F. DE, CRAMAROSSA, L., TONELLI, S., BENEDETTI, G.A., NEGRI, M., BASCHIERI, L., CASSANO, C.: Iodine deficiency in two endemic goiter areas of central and southern Italy. J. clin. Endocr. **26**, 393 (1966).

MALAMOS, B., KOUTRAS, D.A., RIGOPOULOS, G.A., PAPAPETROU, P.D., GOUGAS, E., KELPERI, H., MORAITOPOULOS, C., DAVI, E., LEONARDOPOULOS, J.: Endemic goiter in Greece: Some new epidiomologic studies. J. clin. Endocr. **32**, 130 (1971).

MARINE, D., KIMBALL, O.P.: Prevention of simple goiter in man. Fourth paper. Arch. intern. Med. **25**, 661 (1920).

MATOVINIVIC, J., RAMALINGASWAMI, V.: Therapy and prophylaxis of endemic goitre. In: Endemic goitre, p. 385. Geneva: World Health Organization 1960.

MCCARRISON, R.: Observations on endemic cretinism in the Chitral and Gilgit valleys. Lancet **1908 II**, 1275.

Medical Research Council Goitre Subcommittee. Endemic goitre in England. Lancet **1944 I**, 107.

MERKE, F.: Weshalb unser Kochsalz am 1. Sept. 1962 endlich höher jodiert wurde. Praxis **53**, 1388 (1964).

— Die Eiszeit als primordiale Ursache des endemischen Kropfes. Schweiz. med. Wschr. **95**, 1183 (1965).

— Weitere Belege für die Eiszeit als primordiale Ursache des endemischen Kropfes: Eiszeit und Kropf im Wallis. Schweiz. med. Wschr. **97**, 131 (1967).

— Führt die starke Zunahme des Meerfischkonsums in der Schweiz zu einer neuen „Jodquelle"? Schweiz. med. Wschr. **98**, 1535 (1968).

— Geschichte und Ikonographie des endemischen Kropfes und Kretinismus. Bern: Huber 1971.

MURRAY, I.P.C., STEWART, R.D.H.: Iodide goitre. Lancet **1967 I**, 922.

MYANT, N.B.: Passage of thyroxine and triiodothyronine from mother to foetus in pregnant women. Clin. Sci. **17**, 75 (1958).

— The thyroid and reproduction in mamals. In: R. PITT-RIVERS and W.R. TROTTER, The thyroid gland I. London: Butterworths 1964.

NEGRI, M.: Endemic and congenital goiter. In: Current topics in thyroid research. New York: Academic Press Inc. 1965.

ODDIE, T.H., FISHER, D.A., MCCONAHEY, W.M., THOMPSON, C.S.: Iodine intake in the United States. A reassessment. J. clin. Endocr. **30**, 659 (1970).

PELTOLA, P.: The goitrogenic effect of milk obtained from the region of endemic goitre in Finland. In: Advances in thyroid research, ed. by R. PITT-RIVERS and J.R. TATA, p. 10. New York: Pergamon Press 1961.

PEREZ, C., SCRIMSHAW, N.S., MUÑOZ, J.A.: Technique of endemic goiter surveys. In: Endemic goiter, p. 369. Geneva: WHO 1960.

PHAROA, P.O.D., BUTTFIELD, I.H., HETZEL, B.S.: Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. Lancet **1971 I**, 308.

PODOBA, J., LANGER, P.: Naturally occurring goitrogens and thyroid function. Bratislava: Publ. House Slovak Acad. Sci. 1964.

— STUKOVSKY, R.: Thyroid function after stopping iodinated salt distribution. Acta Endocr. Panam. **3**, 135 (1972).

PRETELL, E.A.: The optimal program for prophylaxis of endemic goiter with iodized oil. In: Human development and the thyroid gland. Relation to endemic cretinism (J.B. STANBURY, R.L. KROC, eds.), p. 267. New York: Plenum Press 1972.

QUERIDO, A.: History of iodine prophylaxis with regard to cretinism and deaf-mutism. In: Adv. in exptl. Biol. Med., vol. 30, Human development and the thyroid gland (J.B. STANBURY, ed.), p. 191. New York: Plenum Press 1972.

QUERVAIN, F. DE, WEGELIN, C.: Der endemische Kretinismus. Berlin u. Wien: Springer 1936.

RAMALINGASWAMI, V., SUBRAMANIAN, T.A.V.: The aetiology of Himalayan endomic goiter. Lancet **1961 I**, 791.

RAMAN, G., BEIERSWALTES, W.H.: Correlation of goiter, deaf-mutism and mental retardation with serum thyroid hormone levels in noncretinous inhabitants of a severe endemic goiter area in India. J. clin. Endocr. **19**, 228 (1959).

RANDOLPH, J., GRUNT, J.A., VAROTER, G.F.: The medical and surgical aspects of intratracheal goiter. New Engl. J. Med. **268**, 457 (1963).

REINWEIN, D.: Über die Pathogenese der Struma. Dtsch. med. Wschr. **88**, 2493 (1963).

— Hormonsynthese und Enzymspektrum bei Erkrankungen der menschlichen Schilddrüse. Acta endocr. (Kbh.), Suppl. 94 (1964).

— HORSTER, F.A.: Die enthyreoten Strumen. Testosteron-Schilddrüse. 13. Symp. Dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.

— KLEIN, E.: Der Einfluß des anorg. Blutjodes auf den Jodumsatz blander Strumen. Acta endocr. (Kbh.) **39**, 328 (1962).

RICCABONA, G.: Die endemische Struma im Tirol. Acta endocr. (Kbh.) **55**, 545 (1967).

— Die endemische Struma. München: Urban & Schwarzenberg 1972.

ROCHE, J., LISSITZKY, S.: Etiology of endemic goitre. In: Endemic goitre, Geneva: World Health Organization 1960.

ROCHE, M.: Elevated thyroidal I^{131} uptake in the absence of goiter in isolated Venezuelan indians. J. clin. Endocr. **19**, 1440 (1959).

— PERINETTI, H., BARBEITO, A.: Urinary excretion of stable iodine in a small group of isolated Venezuelan Indians. J. clin. Endocr. **21**, 1009 (1961).

RÖSLER, H., HORST, W., SCHNEIDER, C., BRUNNER, E.: Die Radiojodresektion der euthyreoten Struma. In: Radio-Isotope in der Endokrinologie. Stuttgart: Schattauer 1965.

SCHOU, M., AMDISEN, A., ESKJAER JENSEN, S., OLSEN, T.: Occurrence of goiter during lithium treatment. Brit. med. J. **1968 III**, 710.

SRINIVASAN, S., SUBRAMANYAN, T.A.V., SINTRA, A., DEO, M.G., RAMALINGASWAMI, V.: Himalayan endemic deaf-mutism. Lancet **1964 II**, 176.

STANBURY, J. B.: A survey of some recent studies on the problem of endemic goitre. In: Radioisotopes in tropical medicine. Vienna: Int. Atomic Energy Agency 1962.
— BROWNELL, G. L., RIGGS, D. S., PERINETTI, H., ITOIZ, J., DEL CASTILLO, E. B.: Endemic goiter. The adaptation of man to iodine deficiency. Cambridge, Mass.: Harvard Univ. Press 1954.
— KROC, R. L. (Eds.): Human development and the thyroid gland. Relation to endemic cretinism. Advanc. exp. biol. Med., vol. 30. New York: Plenum 1972.
— QUERIDO, A.: Genetic and environmental factors in cretinism: a classification. J. clin. Endocr. **16**, 1522 (1956).
STEINER, H., RICCABONA, G., BAUMGARTL, E., LUGGER, L. J.: Probleme der medikamentösen Strumarezidiv-Prophylaxe. Münch. med. Wschr. **10**, 569 (1969).
STUDER, H.: The regulation of thyroid function in iodine deficiency. Bern: Huber 1968.
— GREER, M. A.: A study of the mechanisms involved in the production of iodine-deficiency goiter. Acta endocr. (Kbh.) **49**, 610 (1965).
— KOHLER, H., BÜRGI, H.: Iodine deficiency. Handbook Series of the American Physiologic Society, Volume "Thyroid". Ed. by D. H. SOLOMON and M. A. GREER (1974, in press).
SUZUKI, H., HIGUCHI, T., SAWA, K., OHTAKI, S., HORINCHI, V.: Endemic coast goitre in Hokaido, Japan. Acta endocr. (Kbh.) **50**, 161 (1965).
TAYLOR, S.: Calcium as a goitrogen. J. clin. Endocr. **14**, 1412 (1954).
TAYLOR, S.: The size of follicles in nontoxic goitre. Lancet **1952 I**, 1975.
TERPSTRA, J., QUERIDO, A.: Endemic goitre in the Netherlands. Acta endocr. (Kbh.) **31**, 433 (1959).
TROTTER, W. R.: The association of deafness with thyroid dysfunction. Brit. med. Bull. **16**, 92 (1960).
VISSCHER, M. DE, BECKERS, C., SCHRIECK, H. G. VAN DEN, SMET, M. DE, ERMANS, A. M., GALPERIN, H., BASTENIE, P. A.: Endemic goiter in the Uele Region (Republic of Congo). I. General aspects and functional studies. J. clin. Endocr. **21**, 175 (1961).
VOEGELI, E.: Anatomische Kontrolle der Jod-Prophylaxe des endemischen Kropfes im Kanton Bern 1957–1961. Dissertation. Bern, 1963.
WALTHARD, B.: Über den derzeitigen Stand der Jodsalzprophylaxe des endemischen Kropfes in der Schweiz. Wien. med. Wschr. **112**, 389 (1962).
WARREN, S., MEISSNER, W. A.: Tumors of the thyroid gland. Atlas of tumor pathology, Sect. 4, Fasc 14. Washington: A. F. I. P. 1953.
WAYNE, E. J., KOUTRAS, D. A., ALEXANDER, W. D.: Clinical aspects of iodine metabolism. Oxford: Blackwell 1964.
WELLBY, M. L., POWELL, K., CARMAN, M., HETZEL, B. S.: Comparative studies of diiodotyrosine deiodinase activities in endemic goiter and congenital goiter. J. clin. Endocr. **35**, 762 (1972).
WESPI, H. J.: Die Kropfprophylaxe. Ergebn. inn. Med. Kinderheilk. **61**, 489 (1942).
— Abnahme der Taubstummheit in der Schweiz als Folge der Kropfprophylaxe mit jodiertem Kochsalz. Schweiz. med. Wschr. **75**, 625 (1945).
— 40 Jahre Kropfprophylaxe mit jodiertem Salz. Bull. eidg. Gesundh.-Amt. **2**, 1 (1962).
— The influence of time in iodine prophylaxis. Rev. europ. Endocr. **3**, 337 (1966).
— Kropfprophylaxe mit jodiertem Kochsalz in der Schweiz. Bull. eidg. Gesundh.-Amt **4**, 153 (1968).
WHO: Endemic goitre. Geneva: World Health Org. 1960.
WOLFF, J.: Iodide goiter and the pharmacologic effects of excess iodide. Amer. J. Med. **47**, 101 (1969).

Sporadic Euthyroid Goiter

AGERBAEK, H.: Non-toxic goiter. Dyshormonogenesis. Acta endocr. (Kbh.) **72**, 671 (1973).
BERGFELT, G., RISHOLM, L.: Postoperative thyroid hormone therapy in nontoxic goitre. Acta chir. scand. **126**, 531 (1963).
CASSIDY, C. E., EDDY, R. L.: Hypothyroidism in patients with goiter. Metabolism **19**, 751 (1970).
ERMANS, A. M., KINTHAERT, J., CAMUS, M.: Defective intrathyroidal iodine metabolism in nontoxic goiter. Inadequate iodination of thyroglobulin. J. clin. Endocr. **28**, 1307 (1968).
GREER, M. A.: The natural occurrence of goitrogenic agents. Recent Progr. Hormone Res. **18**, 187 (1962).
LANGER, P., GREER, M. A.: Antithyroid activity of some naturally occurring isothiocyanates *in vitro*. Metabolism **17**, 596 (1968).
LEMARCHAND-BÉRAUD, T., SCAZZIGA, B. R., VANNOTTI, A.: Plasma thyrotropin levels in thyroid diseases and effect of treatment. Acta endocr. (Kbh.) **62**, 593 (1969).
Medical Research Council Goitre Sub-Committe: Endemic goitre in England. Lancet **1944 I**, 107.
RAPOPORT, B., NIEPOMNISZCZE, H., BIGAZZI, M., HATI, R., DE GROOT, L. J.: Studies on the pathogenesis of poor thyroglobulin iodination. J. clin. Endocr. **34**, 822 (1972).
TUBIANA, M., FRAGU, P., DE TOVAR, G., BAZIN, J. P.: The radioactive iodine uptake and the absolute iodine uptake in euthyroid subjects with and without sporadic goiter. Rev. europ. Étud. clin. et biol. **16**, 250 (1971).

Thyroiditis

General Reviews

BASTÉNIE, P. A., ERMANS, A. M.: Thyroiditis and thyroid function. Clinical, morphological and physiopathological studies. Oxford: Pergamon Press 1972.
HARLAND, W. A., FRANTZ, V. K.: Clinico-pathologic study of 261 surgical cases of socalled "thyroiditis". J. clin. Endocr. **16**, 1433 (1956).
WERNER, S. C.: Classification of thyroid disease. J. clin. Endocr. **29**, 860 (1969).

Acute and Subacute Nonsuppurative (De Quervain's) Thyroiditis

BERGEN, S. S., JR.: Acute nonsuppurative thyroiditis. Arch. intern. Med. **102**, 747 (1958).
CZERNIAK, P., HARELL-STEINBERG, A.: The chronology of events in the development of subacute thyroiditis, studied by radioactive iodine. J. clin. Endocr. **17**, 1448 (1957).
EYLAN, E., ZMUCKY, R., SHEBA, CH.: Mumps virus and subacute thyroidits. Lancet **1957 I**, 1062.
GREENE, J. N.: Subacute thyroiditis. Amer. J. Med. **51**, 97 (1971).
HINTZE, G., FORTELINS, P., RAILO, J.: Epidemic thyroiditis. Acta endocr. (Kbh.) **45**, 381 (1964).
HORST, W.: Radiologische Diagnostik und Therapie von benignen Schilddrüsenerkrankungen bzw. Tumoren. Strahlenforschung und Strahlenbehandlung. III. München u. Berlin: Urban & Schwarzenberg 1962.
INGBAR, S. H., FREINKEL, N.: Thyroid function and the metabolism of iodine in patients with subacute thyroidits. Arch. intern. Med. **101**, 339 (1958).
IVY, H. K.: Permanent myxedema: an unusual complication of granulomatous thyroidits. J. clin. Endocr. **21**, 1384 (1961).
LAMBERG, B. A., HINTZE, G., JUSSIBAR, R., BERLIN, M.: Subacute thyroiditis. Acta endocr. (Kbh.) **33**, 457 (1960).
LINDSAY, S., DAILEY, M. E.: Granulomatous or giant cell thyroiditis. Surg. Gynec. Obstet. **98**, 197 (1954).
PERSON, P. S.: Cytodiagnosis of thyroiditis. Acta med. scand., Suppl. 483, (1968).

QUERVAIN, F. DE: Zur Klinik und pathologischen Anatomie der nicht eitrigen Thyreoiditis. Schweiz. med. Wschr. **1936**, 1174.
— Zur Entstehung der akuten bis chronischen nichteitrigen Thyreoiditis. Schweiz. med. Wschr. **1938**, 815.
— GIORDANENGO, G.: Die akute und subakute nichteitrige Thyreoiditis. Mitt. Grenzgeb. Med. Chir. **44**, 538 (1936).
SWANN, N.H.: Acute thyroiditis. Five cases associated with adenovirus infection. Metabolism **13**, 908 (1964).
VAGENAKIS, A.G., ABREAU, C.M., BRAVERMAN, L.E.: Prevention of recurrence in acute thyroiditis following corticosteroid withdrawal. J. clin. Endocr. **31**, 705 (1970).
VANDERLINDE, R.J., MILNE, J.: Subacute thyroiditis. J. Amer. med. Ass. **173**, 1799 (1960).
VOLPÉ, R., JOHNSTON, M.W., HUBER, N.: Thyroid function in subacute thyroiditis. J. clin. Endocr. **18**, 65 (1958).
WEBER, M.: La thyroïdite aseptique de de Quervain. Praxis **52**, 294 (1963).
WERNER, S.C.: ACTH and Cortisone therapy of acute nonsuppurative (subacute) thyreoiditis. J. clin. Endocr. **13**, 1332 (1953).
WOOLNER, L.B., MCCONAHEY, W.M., BEAHRS, O.H.: Granulomatous thyroiditis (de Quervain's thyroiditis). J. clin. Endocr. **17**, 1201 (1957).

Chronic Lymphocytic (Hashimoto's) Thyroiditis

ARDEMAN, S., CHANARIN, J., KRAFCHICK, B., SINGER, W.: Addisonian pernicious anemia and intrinsic factor antibodies in thyroid disorders. Quart. J. Med. N.S. **35**, 421 (1966).
BALFOUR, B.M., DONAICH, D., ROITT, I.M., COUCHMAN, K.G.: Fluorescent antibody studies in human thyroiditis: Autoantibodies to an antigen of thyroid colloid distinct from thyroglobulin. Brit. J. exp. Path. **42**, 307 (1961).
BECKER, K.L., FERGUSON, R.H., MCCONAHEY, W.M.: Connective tissue diseases and symptoms associated with Hashimoto's thyroiditis. New Engl. J. Med. **268**, 277 (1963).
BLIZZARD, R.M., HUNG, W., CHANDLER, R.W., ACETO, T., JR., KYLE, M., WINSHIP, T.: Hashimoto's thyroiditis—Clinical and laboratory response to prolonged cortisone therapy. New Engl. J. Med. **267**, 1015 (1962).
BRAVERMAN, L.E., INGBAR, S.H., VAGENAKIS, A.G., ADAMS, L., MALOOF, F.: Enhanced susceptibility to iodide myxedema in patients with Hashimoto's disease. J. clin. Endocr. **32**, 515 (1971).
BROWN, H., MCGARITY, W.C.: Chronic thyroiditis in childhood. J. Amer. med. Ass. **171**, 1182 (1959).
BUCHANAN, W.W., KOUTRAS, D.A., ALEXANDER, W.D., CROOKS, J., RICHMOND, M.H., MACDONALD, E.M., WAYNE, E.J.: Iodine metabolism in Hashimoto's thyroidits. J. clin. Endocr. **21**, 806 (1961).
MCG. HARDEN, R., KOUTRAS, D.A., GRAY, K.G.: Abnormalities of iodine metabolism in euthyroid nongoitrous women with complement-fixing antimicrosomal thyroid auto-antibodies. J. clin. Endocr. **25**, 301 (1965).
CHERTOW, B.C., FIDLER, W.J., FARISS, B.L.: Graves' disease and Hashimoto thyroiditis in monozygous twins. Acta endocr. (Kbh.) **72**, 18 (1973).
DAILEY, M.E., LINDSAY, S., SKAHEN, R.: Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. Arch. Surg. **70**, 291 (1955).
DECOURT, J., EYQUEM, A., CALMETTES, C., LOUCHART, J.: Sur un cas de goitre de Hashimoto consécutif à une thyroïdite ourlienne. Mise en évidence d'auto-anticorps. Bull. Soc. méd. Hôp. Paris **22**, 773 (1959).
DONIACH, D.: Auto-immune thyroiditis. London: International Academy of Pathology 1964.
— HUDSON, R.V., ROITT, I.M.: Human auto-immune thyroiditis. Clinical studies. Brit. med. J. **1960 I**, 365.
— ROITT, I.M.: Family studies on gastric autoimmunity. Proc. roy. Soc. Med. **59**, 691 (1966).
— — BENHAMOU-GLYNN, N., JAYSON, M.I.V., EL KABIR, D.J.: Thyrotoxicosis and non-toxic Hashimoto goiter in monozygotic twins. In: IRVINE, W.J., Thyrotoxicosis. Edinburgh-London: Livingstone 1967.
Editorial: Auto-immune thyroid disease. Brit. med. J. **1960 I**, 407.
EL KABIR, D.J., DONIACH, D., TURNER-WARWICK, R.: Serum content of thyrotrophic hormone in human thyroiditis: low TSH levels with high radioiodine uptake in mild autoimmune thyroidits. J. clin. Endocr. **23**, 510 (1963).
FATOURECHI, V., MCCONAHEY, W.M., WOOLNER, L.B.: Hyperthyroidism associated with histologic Hashimoto's thyroditis. Mayo Clin. Proc. **46**, 682 (1971).
FEDERLIN, K., OPPERMANN, W., PFEIFFER, E.F.: Beiträge zur Immunthyreoiditis. I. Serologische und fluoreszenzmikroskopische Untersuchungen bei der Hashimoto-Thyreoiditis. Dtsch. med. Wschr. **90**, 247 (1965).
FUJIMOTO, Y., SUZUKI, H., ABE, K., BROOKS, J.R.: Autoantibodies in malignant lymphoma of the thyroid. New Engl. J. Med. **276**, 380 (1967).
FURSZYFER, J., KURLAND, L.T., MCCONAHEY, W.M., WOOLNER, L.B., ELVEBACK, L.R.: Epidemiologic aspects of Hashimoto's thyroiditis and Graves' disease in Rochester, Minnesota (1935–1967), with special reference to temporal trends. Metabolism **21**, 197 (1972).
GHARIB, H., WAHNER, H.W., MCCONAHEY, W.M.: Serum levels of thyroid hormones in Hashimoto's thyroiditis. Mayo Clin. Proc. **47**, 175 (1972).
GROOT, L.J. DE, HALL, R., MCDERMOTT, W.V., JR., DAVIS, A.M.: Hashimoto's thyroiditis, a genetically conditioned disease. New Engl. J. Med. **267**, 267 (1962).
HAFERKAMP, O.: Autoantikörperbedingte Thyreoditis. Dtsch. med. Wschr. **88**, 1275 (1963).
HALL, R., SAXENA, K.M., OWEN, S.G.: A study of the parents of patients with Hashimoto's disease. Lancet **1962 II**, 1291.
HAMLIN, E., JR., VICKERY, A.L.: Needle biopsy of the thyroid gland. New Engl. J. Med. **254**, 742 (1956).
JAYSON, M.I.V., DONIACH, D., BENHAMOU-GLYNN, N., ROITT, I.M., ELKABIR, D.J.: Thyrotoxicosis and Hashimoto goitre in a pair of monozygotic twins with serum long-acting thyroid stimulator. Lancet **1967 II**, 15.
LINDSAY, S.M., DAILY, E.: Malignant lymphoma of the thyroid and its relation to Hashimoto's disease. A clinical and pathological study of 8 patients. J. clin. Endocr. **15**, 1332 (1955).
— FRIEDLANDER, J., YCE, G., SOLEY, M.H.: Chronic thyroiditis: A clinical and pathologic study of 354 patients. J. clin. Endocr. **12**, 1578 (1952).
MASI, A.T., HARTMANN, W.H., HAHN, B.H., ABBEY, H., SHULMAN, L.E.: Hashimoto's disease. A clinicopathological study with matched controls. Lancet **1965 I**, 123.
MCCONAHEY, W.M., KEATING, F.R., JR., BEATUS, O.H., WOOLNER, L.B.: On the increasing occurrence of Hashimotos thyroidits. J. clin. Endocr. **22**, 542 (1962).
MORGANS, M.E., TROTTER, W.R.: Defective organic binding of iodine by the thyroid in Hashimoto's thyroiditis. Lancet **1957 I**, 553.
MORI, T., KRISS, J.P.: Measurements by competitive binding radioassay of serum anti-microsomal and antithyroglobulin antibodies in Graves' disease and other thyroid disorders. J. clin. Endocr. **33**, 688 (1971).
MULHERN, L.M., MASI, E.M., SHULMAN, L.E.: Hashimoto's disease. Lancet **1966 II**, 508.
MURRAY, I.P.C., MCGIRR, E.M.: Radioactive iodine studies in the diagnosis of Hashimoto's thyroiditis. Brit. med. J. **1960 I**, 838.
NÈVE, P., ERMANS, A.M., BASTÉNIE, P.A.: Struma lymphomatosa (Hashimoto). In: P.A. BASTÉNIE, A.M. ERMANS thyroiditis and thyroid function, p. 109. Oxford: Pergamon Press 1972.

OWEN, C.A.: A review of auto-immunization in Hashimoto's disease. J. clin. Endocr. **18**, 1015 (1958).
PAPAPETROU, P.D., LAZARUS, J.H., MCSWEEN, R.N., MCG. HARDEN, R.: Long-term treatment of Hashimoto's thyroiditis with thyroxine. Lancet **1972 II**, 1045.
PERLOFF, W.H.: Thyrotoxicosis following acute thyroiditis. A report of 5 cases. J. clin. Endocr. **16**, 542 (1956).
PIRIBAUER, J., KEMINGER, K.: Zur Pathologie und Klinik der Struma lymphomatosa. Dtsch. med. Wschr. **85**, 1166 (1960).
SCAZZIGA, B.R.: Thyroïdite auto-immune. Helv. med. Acta, Suppl. **46**, 42 (1967).
— LEMARCHAND-BÉRAUD, T., VANNOTTI, A.: Aspects cliniques et biologiques de la thyroïdite chronique auto-immunitaire. Schweiz. med. Wschr. **95**, 897 (1965).
SCHILLER, K.F.T., SNYDER, F.M., VALLOTTON, M.B.: Gastric pathological and immunological abnormalities in Hashimoto's thyroiditis. Gut **8**, 582 (1967).
SHULMAN, L.E., HARVEY, A.M.: Hashimoto's thyroiditis in false-positive reactors to the test for syphilis. Amer. J. Med. **36**, 174 (1964).
STUART, A.E., ALLAN, W.S.A.: The significance of basement-membrane changes in thyroid disease. Lancet **1958 II**, 1204.
VICKERY, A.L., HAMLIN, E.: Struma lymphomatosa (Hashimoto's thyroiditis). Observations on repeated biopsies in 16 patients. New Engl. J. Med. **264**, 226 (1961).
VOLPÉ, R., ROW, V.V., WEBSTER, B.R., JOHNSTON, W., EZRIN, C.: Studies of iodine metabolism in Hashimoto's thyroiditis. J. clin. Endocr. **25**, 593 (1965).
WAKSMAN, B.H.: Auto-immunization and the lesions of autoimmunity. Medicine (Baltimore) **41**, 93 (1962).
WALSER, A., IVANKOVIC, M., BAUR, M.: Sjögren-Syndrom und Struma lymphomatosa Hashimoto. Schweiz. med. Wschr. **95**, 763 (1965).
WOOLNER, L.B., MCCONAHEY, M.W., BEAHRS, O.H.: Struma lymphomatosa (Hashimoto's thyroiditis) and related disorders. J. clin. Endocr. **19**, 53 (1959).

Chronic Invasive Fibrous (Riedel's) Thyroiditis

BARTHOLOMEW, L.G., CAINT, J.C., WOOLNER, B., UTZ, D.C., FERRIS, D.O.: Sclerosing cholangitis. Its possible association with Riedel's struma and fibrous retroperitonitis. Report of 2 cases. New Engl. J. Med. **269**, 8 (1963).
BASTÉNIE, P.A., ERMANS, A.M.: Thyroiditis and thyroid function. Clinical, morphological and physiopathological studies. Oxford: Pergamon press 1972.
GOETSCH, E., KAMNER, M.: Chronic thyroiditis and Riedel's struma: Etiology and pathogenesis. J. clin. Endocr. **15**, 1010 (1955).
HARDMEIER, TH., HEDINGER, CHR.: Beziehungen zwischen der retroperitonealen Fibrose und der sog. Takayasuschen Arteriitis. Schweiz. med. Wschr. **94**, 1669 (1964).
— — Die eisenharte Struma Riedel — eine primäre Gefäßerkrankung? Virchows Arch. path. Anat. **337**, 547 (1964).
RAPHAEL, H.A., BEAHRS, O.H., WOOLNER, L.B., SCHOLZ, D.A.: Riedel's struma with fibrous mediastinitis. Proc. Mayo Clin. **41**, 375 (1966).
WOOLNER, L.B., MCCONAHEY, W.M., BEAHRS, O.H.: Invasive fibrous thyroiditis (Riedel's struma). J. clin. Endocr. **17**, 202 (1957).

Thyroid Neoplasia

Benign Thyroid Tumors and the Solitary Nodule

DE GROOT, L.J.: Lack of iodide trapping in "cold" thyroid nodules. Acta endocr. Panam **1**, 27 (1970).
DE RUBERTIS, F., YAMASHITA, K., DEKKER, A., LARSEN, P.R., FIELD, J.B.: Effects of thyroid stimulating hormone on adenyl cyclase activity and intermediary metabolism of "cold" thyroid nodules and normal thyroid tissue. J. clin. Invest. **51**, 1109 (1972).
Editorial: Thyroid nodules: Thyroid cancer. Lancet **1964 II**, 129.
Editorial: The solitary thyroid nodule. Brit. med. J. **1971 III**, 720.
FUJIMOTO, Y., OKA, A., NAGATAKI, S.: Occurrence of papillary carcinoma in hyperfunctioning thyroid nodule. Report of a case. Endocr. jap. **19**, 371 (1972).
GUINET, P., TOUNIAIRE, J., GUILLAUD, M., BRIERE, J., DALMAIS, J., CHALENDAR, D.: Adénome toxique et cancer thyroïdien. Ann. Endocr. (Paris) **32**, 513 (1971).
KENDALL, L.W., CONDON, R.E.: Prediction of malignancy in solitary thyroid nodules. Lancet **1969 I**, 1071.
MILLER, J.M.: Carcinoma and thyroid nodules. The problem in an endemic goiter area. New Engl. J. Med. **252**, 247 (1955).
MURRAY, I.P.C., STEWART, R.D.H., INDYK, J.S.: Thyroid scanning with ^{131}Cs. Brit. med. J. **1970 IV**, 653.
TAYLOR, S., PSARRAS, A.: The solitary thyroid nodule- benign or malignant. Praxis **56**, 370 (1967).
VEITH, F.J., BROOKS, J.R., GRIGSBY, W.P., SELENKOW, H.A.: The nodular thyroid gland and cancer. A practical approach to the problem. New Engl. J. Med. **270**, 431 (1964).
WELCH, C.E.: Therapy for multinodular goiter. J. Amer. med. Ass. **195**, 339 (1966).

Malignant Thyroid Tumors

ALHADEFF, R., SCOTT, F., TAYLOR, S.: A clinico-pathological study of thyroid carcinoma. Brit. J. Surg. **43**, 617 (1955/56).
BLACK, B.M., KIRK, T.A., JR., WOOLNER, L.B.: Multicentricity of papillary adenocarcinoma of the thyroid: Influence of treatment. J. clin. Endocr. **20**, 130 (1960).
BLAHD, W.H., NORDYKE, R.A., BAUER, F.K.: Radioactive iodine I^{131} in the postoperative treatment of thyroid cancer. Cancer (Philad.) **13**, 745 (1960).
BRUNNER, K.: Schilddrüsenkarzinom im Kindesalter nach Röntgenbestrahlung eines Naevus vasculosus cutaneus vor 12 Jahren. Schweiz. med. Wschr. **91**, 389 (1961).
BUCKWALTER, J.A.: Age and thyroid carcinoma. Arch. Surg. **82**, 916 (1961).
BURN, J.I., TAYLOR, S.F.: Natural history of thyroid carcinoma. Brit. med. J. **1962 II**, 1218.
CHESKY, V.E., DREESE, W.C., HELLWIG, C.A.: Haemangioendothelioma of the thyroid. J. clin. Endocr. **13**, 801 (1953).
CONARD, R.A., DOBYNS, B.M., SUTOW, W.W.: Thyroid neoplasia from radioactive fallout. J. Amer. med. Ass. **214**, 316 (1970).
— RALL, J.E., SUTOW, W.W.: Thyroid nodules as a late sequela of radioactive fallout. New Engl. J. Med. **274** 1391 (1966).
CRILE, G., JR.: Late results of treatment for papillary cancer of the thyroid. Ann. Surg. **160**, 178 (1964).
— Endocrine dependence of papillary carcinomas of the thyroid. J. Amer. med. Ass. **195**, 721 (1966).
CRILE, J.R.G., JR., HAZARD, J.B.: Incidence of cancer in struma lymphomatosa. Surg. Gynec. Obstet. **115**, 101 (1962).
DONAHOWER, G.F., SCHUMACHER, O.P., HAZARD, J.B.: Medullary carcinoma of the thyroid—a cause of Cushing's syndrome: report of two cases. New Engl. J. Med. **28**, 1199 (1968).
DUFFY, B.J.: Can radiation cause thyroid cancer? J. clin. Endocr. **17**, 1383 (1957).
EGLOFF, B.: Bösartige Schilddrüsengeschwülste mit besonderer Berücksichtigung maligner Rezidive primär gutartiger Kröpfe. Schweiz. med. Wschr. **91**, 424 (1961).
— HEDINGER, CHR.: Zum Begriff der wuchernden Struma Langhans. Schweiz. med. Wschr. **94**, 1417 (1964).
Editorial: Phaeochromocytoma and thyroid cancer. Brit. med. J. **1965 II**, 549.

FEDERMAN, D.D.: Hyperthyroidism due to functioning metastatic carcinoma of the thyroid. Medicine (Baltimore) **43**, 267 (1964).
FRANSSILA, K.: Value of classification of thyroid cancer. Acta path. microbiol. scand., Suppl. 225 (1971).
FRAZELL, E.L., FOOTE, F.W.: Papillary cancer of the thyroid. Cancer (Philad.) **11**, 895 (1958).
GERARD-MARCHANT, R.: Thyroid follicle inclusions in cervical lymph nodes. Arch. Path. **77**, 633 (1964).
— PILARD, J.D., BABINET, J., CASQUET, C.: Les calcifications thyroïdiennes. Presse méd. **70**, 1849 (1962).
GOOLDEN, A.W.G.: Carcinoma of the thyroid following irradiation. Brit. med. J. **1958II**, 954.
GRICOUROFF, G.: La thyroïdose métastatique bénigne et ses tumeurs. Bull. Ass. franç. Cancer **49**, 300 (1962).
HAYNE, TH.P., NOFALL, M.M., BEIERWALTES, W.H.: Treatement of thyroid carcinoma with I^{131}. J. Amer. med. Ass. **183**, 303 (1963).
HAZARD, J.B., HAWK, W.A., CRILE, G., JR.: Medullary (solid) carcinoma of the thyroid. J. clin. Endocr. **19**, 152 (1959).
HEDINGER, CHR.: Normale und pathologische Anatomie der Schilddrüse. Verh. dtsch. Ges. inn. Med. **66**, 13 (1960).
— CORBAT, F., EGLOFF, B.: Schilddrüsenmetastasen hypernephroider Nierenkarzinome. Schweiz. med. Wschr. **97**, 1420 (1967).
— SOBIN, L.H. et al.: Histological typing of thyroid tumors. Geneva: World health organization 1973.
— Thyroid cancer, UICC Monograph Series, vol. 12. Berlin-Heidelberg-New York: Springer 1969.
HEDINGER, E.: Zur Lehre der Struma sarcomatosa. Die Blutgefäßendotheliome der Struma. Frankfurt. Z. Path. **3**, 487 (1909).
— Über Intima-Sarcomatosa von Venen und Arterien in sarcomatösen Strumen. Virchows Arch. path. Anat. **164**, 199 (1901).
HERRMANN, E.: Die Bedeutung fortgesetzter Thiouracilmedikation für die Proliferation des Schilddrüsengewebes. Schweiz. med. Wschr. **81**, 1097 (1951).
HILL, C.S., CLARK, R.L., WOLF, M.: The effect of subsequent pregnancy on patients with thyroid carcinoma. Surg. Gynec. Obstet. **122**, 1219 (1966).
— IBANEZ, M.L., SAMAAN, N.A., AHEARN, M.J., CLARK, R.L.: Medullary (solid) carcinoma of the thyroid gland: An analysis of the M.D. Anderson Hospital experience with patients with the tumor, its special features, and its histogenesis. Medicine (Baltimore) **52**, 141 (1973).
HILTON, G., POCHIN, E.E., CUNNINGHAM, R.M., HALNAU, K.E.: The role of radioiodine in the treatment of carcinoma of the thyroid. Brit. J. Radiol. **29**, 297 (1956).
HIRABAYASHI, R.N., LINDSAY, S.: Carcinoma of the thyroid gland: statistical study of 390 patients. J. clin. Endocr. **21**, 1596 (1961).
— — Thyroid carcinoma and chronic thyroiditis of the Hashimoto type: A statistical study of their relationship. Int. Coll. Tum. thyr. Gland. Marseilles 1964, p. 272. Basel: Karger 1966.
HORN, R.C.: Hürthle cell tumors of the thyroid. Cancer (Philad.) **7**, 234 (1954).
HORST, W.: Strahlentherapie der Schilddrüsenerkrankungen mit Radiojod I^{131}. Internist (Berl.) **1**, 373 (1960).
— Radiojod in Diagnostik und Therapie der Schilddrüsenneoplasmen. In: H. SCHWIEGK and F. TURBA. Künstliche radioaktive Isotope in Physiologie, Diagnostik und Therapie, 2nd ed. Berlin-Heidelberg-New York: Springer 1961.
HUNT, W.B., CRISPELL, K.R., MCKEE, J.: Functioning metastatic carcinoma of the thyroid producing clinical hyperthyroidism. Amer. J. Med. **28**, 995 (1960).
JACKSON, C.E., TASHJIAN, A.H., BLOCK, M.A.: Detection of medullary thyroid cancer by calcitonin assay in families. Ann. intern. Med. **78**, 845 (1973).
JANOWER, M.L., MIETTINEN, O.S.: Neoplasms after thymus irradiation. J. Amer. med. Ass. **215**, 753 (1971).
JOHNSON, R.W.P.: The so-called lateral aberrant thyroid. Brit. med. J. **1962I**, 1668.
KIND, H.P.: Die Häufigkeit der Struma maligna in Sektions- und Operationsgut des pathologischen Instituts der Universität Zürich von 1900 bis Mitte 1964. Schweiz. med. Wschr. **96**, 560 (1966).
KLINCK, G.H.: Hemangioendothelioma and sarcoma of the thyroid. In: Thyroid cancer, UICC monograph series, vol. 12 (CHR. HEDINGER, ed.), p. 60. Berlin-Heidelberg-New York: Springer 1969.
— WINSHIP, T.: Psammoma bodies and thyroid cancer. Cancer (Philad.) **12**, 656 (1959).
LANGHANS, TH.: Über die epithelialen Formen der malignen Struma. Virchows Arch. path. Anat. **189**, 69 (1907).
LEMARCHAND-BÉRAUD, TH., SCAZZIGA, B.R., VANNOTTI, A.: Etude de la biosynthèse et du transport des hormones thyroïdiennes dans le cancer de la thyroïde. Schweiz. med. Wschr. **96**, 348 (1966).
LINDSAY, S.: Carcinoma of the thyroid gland. Springfield: Ch.C. Thomas 1960.
— DAILEY, M.E.: Malignant lymphoma of the thyroid gland and its relation to Hashimoto disease: A clinical and pathological study of 8 patients. J. clin. Endocr. **15**, 1332 (1965).
LUNDSGAARD-HANSEN, P.: Zur Frage der Bedeutung der Thiouracil-Derivate für die Entstehung maligner Tumoren, insbesondere von Schilddrüsentumoren. Oncologia (Basel) **9**, 33 (1956).
MAJARAKIS, J.D., SLAUGHTER, D.P., COLE, W.H.: Thyroid cancer in childhood and adolescence. J. clin. Endocr. **16**, 1487 (1956).
MARKEY, W.S., RYAN, W.G., ECONOMOU, S.G., SIZEMORE, G.W., ARNAUD, C.: Familial medullary carcinoma and parathyroid adenoma without pheo-chromocytoma. Ann. intern. Med. **78**, 898 (1973).
MARTZ, G., MEIER, E.: Über die Radiojodbehandlung der Struma maligna. Schweiz. med. Wschr. **94**, 852 (1964).
MASSINI, M.A.: Über Sarkome der Schilddrüse mit besonderer Berücksichtigung der Lympho- und Retothelsarkome. Schweiz. Z. Path. **19**, 259 (1956).
MEISSNER, W.A., PHILLIPS, M.J.: Diffuse small-cell carcinoma of the thyroid. Arch. Path. **74**, 291 (1962).
MEISSNER, W.A.: Pathology of the thyroid. In: The thyroid (S.C. WERNER and S.H. INGBAR, ed.), 3rd ed., p. 343. New York: Harper & Row 1971.
MELVIN, K.E.W., MILLER, H.H., TASHJIAN, A.H.: Early diagnosis of medullary carcinoma of the thyroid gland by means of calcitonin assay. New Engl. J. Med. **285**, 1115 (1971).
MEYER, J.S., ABDEL-BARI, W.: Granules and thyro-calcitonine-like activity in medullary carcinoma of the thyroid gland. New Engl. J. Med. **278**, 523 (1968).
MILLER, J.M.: Carcinoma and thyroid nodules. The problem in an endemic goiter area. New Engl. J. Med. **252**, 247 (1955).
NEHER, R., RINIKER, B., RITTEL, W., ZUBER, H.: Menschliches Calcitonin. Struktur von Calcitonin M und D Helv. chim. Acta **51**, 1900 (1968).
NISHIYAMA, R.H., SCHMIDT, R.W., BATSAKIS, J.G.: Carcinoma of the thyroid gland in children and adolescents. J. Amer. med. Ass. **181**, 1034 (1962).
PEARSE, A.G.E.: Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C cells and calcitonin. Proc. roy. Soc. B **170**, 71–80 (1968).
POCHIN, E.E.: What is a permissible dose? Hlth. Phys. **9**, 1091 (1963).
— Prospects from treatment of thyroid carcinoma with radioiodine. Clin. Radiol. **18**, 113 (1967).
RALL, J.E., ALPERS, J.B., LEWALLEN, C.G., SONENBERG, M., BERMAN, M., RAWSON, R.W.: Radiation pneumonitis and fibrosis: A complication of I^{131} treatment of pulmonary

metastases from cancer of the thyroid. J. clin. Endocr. **17**, 1263 (1957).
RAWSON, R. W.: Physiological considerations in the management of thyroid cancer. In: Radioisotope in der Endokrinologie. Stuttgart: Schattauer 1965.
ROONEY, D. R., POWELL, R. W.: Carcinoma of the thyroid in children after X-ray therapy in early childhood. J. Amer. med. Ass. **169**, 1 (1959).
ROSE, R. G., KELSEY, M. P., RUSSEL, W. O., STANER, M. L., WHITHE, E. C., CLARK, R. L.: Follow up study of thyroid cancer healed by unilateral lobectomy. Amer. J. Surg. **106**, 494 (1963).
SAUTER, C.: Psammomkörper in Schilddrüsen eines Kropfendemiegebietes. Virchows Arch. path. Anat. **337**, 328 (1964).
SCHIMKE, R. N., HARTMANN, W. H.: Familial amyloid-producing medullary thyroid carcinoma and phaeochromocytoma. A distinct genetic entity. Ann. intern. Med. **63**, 1027 (1965).
— — PROUT, T. E., RIMOIN, D. L.: Syndrome of bilateral pheochromocytoma, medullary thyroid carcinoma and multiple neuromas. New Engl. J. Med. **279**, 1 (1968).
SHIMAOKA, K., SOKAL, J. E., PICKREN, J. W.: Metastatic neoplasms in the thyroid gland. Cancer (Philad.) **15**, 557 (1962).
SIPPLE, J. H.: The association of pheochromocytoma with carcinoma of the thyroid gland. Amer. J. Med. **31**, 163 (1961).
SISSON, J. C., SCHMIDT, R. W., BEIERWALTERS, W. H.: Sequestered nodular goiter. New Engl. J. Med. **270**, 927 (1964).
SLOAN, L. W.: Of the origin, characteristics and behavior of thyroid cancer. J. clin. Endocr. **14**, 1309 (1954).
SOCOLOW, E. L., HASHIZUME, A., NERIISHI, S., NIITANI, R.: Thyroid carcinoma in man after exposure to ionizing radiation. A summary of the findings in Hiroshima and Nagasaki. New Engl. J. Med. **268**, 406 (1963).
SOLLBERGER, W.: Das großzellige Adenom der Schilddrüse. Schweiz. Z. Path. **20**, 286 (1957).
STEINER, A. L., GOODMAN, A. D., POWER, S. R.: Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease. Multiple endocrine neoplasia, type 2. Medicine (Baltimore) **47**, 371 (1968).
STUDER, H., VERAGUTH, P., WYSS, F.: Thyrotoxicosis due to a solitary hepatic metastasis of thyroid carcinoma. J. clin. Endocr. **11**, 1334 (1961).
TAYLOR, S.: Thyroid carcinoma Postgrad. Med. J. **44**, 404 (1968).
— Non-toxic goiter: sporadic. In: The thyroid. S. C. WERNER and S. H. INGBAR, eds., p. 424. New York: Harper and Row 1971.
— PSARRAS, A.: The solitary thyroid nodule—benign or malignant. Praxis **56**, 370 (1967).
THALMANN, A.: Die Häufigkeit der Struma maligna am Berner Pathologischen Institut von 1910 bis 1950 und ihre Beziehungen zur Jodprophylaxe des endemischen Kropfes. Schweiz. med Wschr. **84**, 473 (1954).
TILL, A. S.: Carcinoma of the thyroid. Proc. roy. Soc. Med. **58**, 309 (1965).
TUBIANA, M., MILHAUD, G., COUTRIS, G., LACOUR, J., PARMENTIER, C., BOK, B.: Medullary carcinoma and thyrocalcitonin. Brit. med. J. **4**, 87 (1968).
UEHLINGER, E.: Nomenclature, p. 64, in: Thyroid cancer, ed. by CHR. E. HEDINGER, UICC Monograph Series, vol. 12. Berlin-Heidelberg-New York: Springer 1969.
VALENTA, L., LEMARCHAND-BÉRAUD, TH., VANNOTTI, A., ŠILNIK, K.: L'hormonogénèse dans le néoplasme thyroïdien. Schweiz. med. Wschr. **98**, 101 (1968).
VARMA, V. M., BEIERWALTES, W. H., NOFAL, M. M., NISHIYAMA, R. H., COPP, J. A.: Treatment of thyroid cancer. J. Amer. med. Ass. **214**, 1437 (1970).
VILLARD, G.: Le sarcome thyroïdien: fréquence et problèmes de diagnostic histologique. Schweiz. med. Wschr. **100**, 369 (1970).
WAHNER, H. W., CUELLO, C., ALJURE, F.: Hormone-induced regression of medullary (solid) thyroid carcinoma. Amer. J. Med. **45**, 789 (1968).
WALNER, H. W., CUELLO, C., CORREA, P., URIBE, L. F., GAITAN, E.: Thyroid carcinoma in an endemic goiter area, Cali, Colombia. Amer. J. Med. **40**, 58 (1966).
WALT, A. J., WOOLNER, L. B., BLACK, B. M.: Primary malignant lymphoma of the thyroid. Cancer (Philad.) **10**, 663 (1957).
WALTHARD, B.: Der Gestaltwandel der Struma maligna in bezug auf die Jodprophylaxe des Kropfes. Schweiz. med. Wschr. **93**, 809 (1963).
WARREN, S., MEISSNER, W. A.: Tumors of the thyroid gland Atlas of tumor pathology, Sect. 4. Fasc. 14, A. F. I. P. Washington 1953.
WEGELIN, C.: Schilddrüse. In: Handbuch der speziellen pathologischen Anatomie und Histologie. O. LUBARSCH, F. HENKE, R. RÖSSLE, E. UEHLINGER (eds.). Berlin: Springer 1926.
WEGMANN, W.: Schilddrüsensarkom nach Bestrahlung im Erwachsenenalter. Schweiz. med. Wschr. **92**, 39 (1962).
WELCH, C. E.: Therapy for multinodular goiter. J. Amer. med. Ass. **195**, 339 (1966).
WELCH, J. W., HELLWIG, C. A.: Multiple malignant tumors of the thyroid. J. int. Coll. Surg. **40**, 492 (1963).
WERNER, S. C.: Classification of thyroid disease. J. clin. Endocr. **29**, 860 (1969).
WILLIAMS, E. D.: A review of 17 cases of carcinoma of the thyroid and phaeochromocytoma. J. clin. Path. **18**, 288 (1965).
— Diarrhoea and thyroid carcinoma. Proc. roy. Soc. med. **59**, 602 (1966).
— Histogenesis of medullary carcinoma of the thyroid. J. clin. Path. **19**, 114 (1966).
— KARIM, S. M. M., SANDLER, M.: Prostaglandin secretion by medullary carcinoma of the thyroid. Lancet **1968 I**, 22.
WINSHIP, TH., ROSVOLL, R. V.: A study of thyroid cancer in children. Amer. J. Surg. **102**, 747 (1961).
WOLFE, H. J., MELVIN, K. E. W., CERVI-SKINNER, S. J., ALSAADI, A. A., JULIAR, J. F., JACKSON, CH. É., TASHJIAN, A. H., JR.: C-cell hyperplasia preceding medullary thyroid carcinoma. New Engl. J. Med. **289**, 437 (1973).

Thyroid Function Tests

LAMBERG, B. A.: Thyroid function tests. Acta endocr. (Kbh.), Suppl. **124**, to Vol. 56, 153 (1967).
SOLOMON, D. H., BENOTTI, J., DE GROOT, L. J., GREER, M. A., PILEGGI, V. J., PITTMAN, J. A., ROBBINS, J., SELENKOW, H. A., STERLING, K., VOLPE, R.: A nomenclature for tests of thyroid hormones in serum: A report of a committee of the American Thyroid Association. J. clin. Endocr. **34**, 884 (1972).
STUDER, H., HUBER, D.: Schilddrüsenfunktionsprüfungen: Diagnostische Ausbeute und Fehlerquellen. Schweiz. med. Wschr. **97**, 1337 (1967).

Serum Thyroxine, T_4

CHOPRA, I. J., SOLOMON, D. H., HO, R. R.: A radioimmunoassay of thyroxine. J. clin. Endocr. **33**, 865 (1971).
MURPHY, B. E. P., PATTEE, C. J.: The measurement of thyroxine in blood. In: Clinical endocrinology II, ed. by E. B. ASTWOOD and C. E. CASSIDY. New York: Grune & Stratton 1968.
MURPHY, B. P.: The determination of thyroxine by competitive protein-binding analysis employing an anion-exchange resin and radiothyroxine. J. Lab. clin. Med. **66**, 161 (1965).

Serum Triiodothyronine, T_3

GHARIB, H., RYAN, R.J., MAYBERRY, W.E.: Triiodothyronine (T_3) radioimmunoassay: A critical evaluation. Mayo Clin. Proc. **47**, 934 (1972).

HORN, K., RUHL, T., SCRIBA, P.C.: Semiautomatic method for the separation and determination of total triiodothyronine and thyroxine in serum. Z. klin. Chem. **10**, 99 (1972).

HÜFNER, M., HESCH, R.-D.: Radioimmunoassay for triiodothyronine in human serum. Acta endocr. (Kbh.) **72**, 464–474 (1973).

LARSEN, P.R.: Direct immunoassay of triiodothyronine in human serum. J. clin. Invest. **51**, 1939 (1972).

PATEL, Y.C., BURGER, H.G.: A simplified radioimmunoassay for triiodothyronine. J. clin. Endocr. **36**, 187 (1973).

SPARAGANA, M., PHILLIPS, G., KUCERA, L.: Serum thyroxine by competitive protein binding analysis: clinical, statistical and comparative evaluation. J. clin. Endocr. **29**, 191 (1969).

STERLING, K., BELLABARBA, D., NEWMAN, E.S., BRENNER, M.A.: Determination of triiodothyronine concentration in human serum. J. clin. Invest. **48**, 1150 (1969).

Percent Free Thyroxine, $\%FT_4$

LIEWENDAHL, K., LAMBERG, B.A.: Free thyroxine in serum determined by dialysis and sephadex-G-25 filtration. J. clin. Endocr. **25**, 991 (1965).

OSORIO, C., JACKSON, D.J., GARTSIDE, J.M., GOOLDEN, A.W.G.: The assessment of free thyroxine in plasma. Clin. Sci. **23**, 525 (1962).

STERLING, K., BRENNER, M.A.: Free thyroxine in human serum: simplified measurement with the aid of magnesium precipitation. J. clin. Invest. **45**, 153 (1966).

Resin T_3 Uptake, RT_3U and Free Thyroxine Index

ASHKAR, F.S., BEZJIAN, A.A.: Use of normalized serum thyroxine. J. Amer. med. Ass. **221**, 1483 (1972).

BLOMSTEDT, B., EINHORN, J.: Effect of cortisone on the PBI and the resin uptake of I^{131}-Triiodothyronine: Metabolism **16**, 319 (1967).

CHRISTENSEN, L.K.: Triiodothyronine uptake by erythrocytes. Acta med. scand. **166**, 141 (1960).

CLARK, F.: Resin uptake of I^{131}-Triiodothyronine. Lancet **1963II**, 167.

— The estimation of thyroid hormone binding by plasma proteins and of unbound levels of thyroxine in plasma. J. clin. Path., Suppl. **20**, 344 (1967).

— HORN, D.B.: Assessment of thyroid function by the combined use of the serum protein-bound iodine and resin uptake of I^{131}-Triiodothyronine. J. clin. Endocr. **25**, 39 (1965).

HAMOLSKY, M.W., GOLODETZ, A., FREEDBERG, A.S.: The plasma protein thyroid hormone complex in man: III. Further studies on the use of the *in vitro* red blood cell uptake of I^{131}-l-triiodothyronine as a diagnostic test in thyroid function. J. clin. Endocr. **19**, 163 (1959).

HORSTER, F.A., KLEIN, E.: Die Anwendung von radioaktivem Trijodthyronin zur Diagnostik der Schilddrüsenfunktion *in vitro*. Dtsch. med. Wschr. **89**, 983 (1964).

IRVINE, W.J., STANDEVEN, R.M.: Serum triiodothyronin uptake using coated charcoal in the assessment of thyroid function. J. Endocr. **41**, 31 (1968).

NAVA, M., GROOT, L.J.DE: Resin uptake of I^{131} labeled triiodothyronine as a test of thyroid function. New Engl. J. Med. **266**, 1307 (1962).

OSORIO, C., JACKSON, D.J., GARTSIDE, J.M., GOOLDEN, A.W.G.: The assessment of free thyroxine in plasma. Clin. Sci. **23**, 525 (1962).

RAGAZ, A., STUDER, H.: Conjunct interpretation of serum ^{131}I-triiodothyronine binding and protein bound iodine as indices of thyroid function in patients with abnormal thyroxine-binding globulin levels. Helv. med. Acta **33**, 460 (1967).

SCRIBA, P.C., HENZE, H.G., LANDGRAF, R., FREY, K.W., SCHWARZ, K.: Klinische Bedeutung der Bestimmung der Bindung von Trijodthyronin an Serumproteine mittels Dextran-Gel-Filtration. Klin. Wschr. **44**, 131 (1966).

STERLING, K., TABACHNIK, M.: Resin uptake of I^{131}-Triiodothyronine as a test of thyroid function. J. clin. Endocr. **21**, 456 (1961).

THORSON, S.C., MINCEY, E.K., MCINTOSH, H.W., MORRISON, R.T.: Evaluation of a new in-vitro blood test for determining thyroid status: the effective thyroxine ratio. Brit. med. J. **1972II**, 67.

YEN, W.: In vitro studies of thyroid function with erythrocyte uptake of I^{131}-labelled-l-Triiodothyronine. Acta endocr. (Kbh.), Suppl. 76 (zu Bd. 42) (1963).

Protein-Bound Iodine, Butanol Extractable Iodine, PBI, BEI

BARKER, S.B., HUMPHREY, M.J., SOLEY, M.H.: The clinical determination of protein-bound iodine. J. clin. Invest. **11**, 91 (1951).

BLACKBURN, CH.M., POWER, M.H.: Diagnostic accuracy of serum protein-bound iodine determination in thyroid disease. J. clin. Endocr. **15**, 1379 (1955).

BLOMSTEDT, B., EINHORN, J.: Effect of cortisone on the PBI and the resin uptake of I^{131} triiodothyronine. Metabolism **16**, 319 (1967).

DANOWSKI, T.S., JOHNSTON, S.Y., PRICE, W.C., MCKELOY, M., STEVENSON, S.S., MCCLUSHEY, E.R.: Protein-bound iodine in infants from birth to one year of age. Pediatrics **7**, 240 (1951).

DAVIS, P.J.: Factors affecting the determination of the serum protein-bound iodine. Amer. J. Med. **40**, 918 (1966).

FOLDENAUER, A., MEYNEN, CH., BÖHN, P.: Über das Verhalten des proteingebundenen Jods (PBI^{127}) im Serum nach Verabreichung jodhaltiger Substanzen, insbesondere Röntgenkontrastmittel. Dtsch. med. Wschr. **92**, 745 (1967).

HOCHULI, E., WEGMANN, T., ROSENMUND, H.: Die Beeinflussung des eiweißgebundenen Jods im Blut durch die Schillersche Jodprobe. Schweiz. med. Wschr. **91**, 1561 (1961).

JONES, J.E., SHULTZ, J.S.: Determination of the thyroxine iodine content of serum contaminated by organic iodines. A method based on gel-filtration. J. clin. Endocr. **27**, 877 (1967).

MAN, E.B., KYDD, D.M., PETERS, J.P.: Butanol extractable iodine of serum. J. clin. Invest. **30**, 531 (1951).

SCHATZ, D.L., VOLPÉ, R.: Lack of diurnal variation in the level of serum protein-bound iodine. J. clin. Endocr. **19**, 1495 (1959).

SÖNKSEN, P.H., EKINS, R.P., STEVENS, H.G., WILLIAMS, E.S., NABARRO, J.D.N.: Serum levels of protein-bound iodine and thyroxine after a course of Clioquinol. Lancet **1968II**, 425.

Radioactive Iodine Uptake

CLARK, F., HALL, R., ORMSTON, B.: Diazepam and tests of thyroid function. Brit. med. J. **1971I**, 585.

CHAPLAN, R.H., GAIDULA, J.J.: Thyroid uptake of radioactive iodine. J. Amer. med. Ass. **215**, 916 (1971).

FISHER, D.A., PANOS, T.C.: "Due caution" and radioiodine in children. Amer. J. Dis. Child. **103**, 729 (1962).

FREUND, G., THOMAS, W.C., JR., BIRD, E.D., KINMAN, R.N., BLACK, A.P.: Effect of iodinated, water supplies on thyroid function. J. clin. Endocr. **26**, 619 (1966).

GRAYSON, R.R.: Factors which influence the radioactive iodine thyroidal uptake test. Amer. J. Med. **28**, 397 (1960).

HORST, W.: Klinische Radiojoddiagnostik der Schilddrüsenerkrankungen. Strahlenbiologie Ergebn. 1952—58. Stuttgart: Thieme 1959.
— Radiologische Diagnostik und Therapie von benignen Schilddrüsenerkrankungen bzw. -tumoren. Strahlenforschung und Strahlenbehandlung, Bd. III. München-Berlin: Urban & Schwarzenberg 1962.
— PETERSEN, I., THIEMANN, KL. J., ZUKSCHWERDT, L.: Methoden und Ergebnisse der Differentialdiagnostik von Schilddrüsenerkrankungen durch die Szintigraphie und das Radiojod-Dreiphasenstudium. Dtsch. med. Wschr. **85**, 711, 728 (1960).
— SAUTTER, H., ULLERICH, K.: Radiojoddiagnostik und Strahlentherapie der endokrinen Ophthalmopathie. Dtsch. med. Wschr. **85**, 730, 805 (1960).
IAEA Panel: Calibration and standardization of thyroid radioiodine uptake measurements. Int. J. appl. Radiat. **17**, 183 (1962).
IAEA Panel: Thyroid radionuclide uptake measurements. Int. J. appl. Radiat. **23**, 305 (1972).
KOHN, L. A., NICHOLS, E. B.: Interference with uptake of radioiodine tracer during the administration of vitamin-mineral mixtures. New Engl. J. Med. **253**, 286 (1955).
LIEWENDAHL, K.: Iodochloroxyquinoline and the thyroid gland. Acta endocr. (Kbh.), Suppl. **133** to Bd. 59 (1968).
LONDON, W. T., VOUGHT, R. L., BROWN, F. A.: Bread — a dietary source of large quantities of iodine. New Engl. J. Med. **273**, 381 (1965).
ODDIE, T. H., GLANIGAN, W. J., FISHER, D. A.: Iodine and thyroxine metabolism in anephric patients receiving chronic peritoneal dialysis. J. clin. Endocr. **31**, 277 (1970).
PITTMAN, J. A., JR., DAILEY, G. E., BESCHI, R. J.: Changing normal values for thyroidal radioiodine uptake. New Engl. J. Med. **280**, 1431 (1969).
RALL, J. E.: The role or radioactive iodine in the diagnosis of thyroid disease. Amer. J. Med. **20**, 719 (1956).
RICCABONA, G.: I^{125} in the clinical evaluation of thyroid disease. New Engl. J. Med. **273**, 126 (1965).
SACKS, B. A., SIEGEL, E., HORWITT, B. N.: Bread iodine content and thyroid radioiodine uptake: A tale of two cities. Brit. med. J. **1972 I**, 79.
SAXENA, K. M., CHAPMAN, E. M., PRYLES, C. V.: Minimal dosage of iodide required to suppress uptake of iodine-131 by normal thyroid. Science **138**, 430 (1962).
SELENKOW, H. A., REFETOFF, S.: Common tests of thyroid function in serum. J. Amer. med. Ass. **202**, 135 (1967).
SILVER, S.: Radioactive isotopes in clinical medicine. New Engl. J. Med. **272**, 466 (1965).
SOENKSEN, P. H., EKINS, R. P., STEVENS, H. G., WILLIAMS, E. S., NABARRO, J. D. L.: Serum-levels of protein-bound iodine and thyroxine after a course of Clioquinol. Lancet **1968 II**, 425.
STECK, A., STECK, B., KOENIG, M. P., STUDER, H.: Auswirkungen einer verbesserten Jodprophylaxe auf Kropfendemie und Jodstoffwechsel. Schweiz. med. Wschr. **102**, 829 (1972).
STUDER, H.: Der TSH-Rervetest mit Carbimazol. Helv. med. Acta **29**, 275 (1962).
— LIPS, B., KOENIG, M. P., BUCHER, H. J., ROHNER, R., KOHLER, H., LEMARCHAND-BÉRAUD, T.: Recovery of initially suppressed thyroidal radioiodine uptake during long-term antithyroid treatment of euthyroid volonteers and hyperthyroid patients. Acta endocr. (Kbh.) **70**, 697 (1972).
THOMAS, I. D., ODDIE, T. H., MYHILL, J.: A diagnostic radioiodine uptake test in patients receiving antithyroid drugs. J. clin. Endocr. **20**, 1601 (1960).

Radioactive Technetium Uptake

GOOLDEN, A. W. G., GLASS, H. I., WILLIAMS, E. D.: Use of $^{99}Tc^{m}$ for the routine assessment of thyroid function. Brit. med. J. **1971 IV**, 396.
VAN T'HOFF, W., POVER, G. G., EISER, N. M.: Technetium-99^{m} in the diagnosis of thyrotoxicosis. Brit. med. J. **1972 III**, 203.

Basal Metabolic Rate

DU BOIS, E. F.: Basal metabolism in health and disease, 3rd ed. Philadelphia: Lea & Febiger 1936.
FLEISCH, A.: Nouvelle méthode d'étude des échanges gazeux et de la fonction pulmonaire. Basel: Benno Schwabe & Co. 1954.
FOWLER, W. S., BLACKBURN, CH. M., HELMHOLTZ, F., JR.: Determination of basal rate of oxygen consumption by open and closed circuit methods. J. clin. Endocr. **17**, 786 (1957).
KARLBERG, P., LIND, J.: Determination of the electrical capacity of the body surface. Acta paediat. (Stockh.) **39**, 454 (1950).
KEATING, F. R.: In defense of the basal metabolic rate. J. clin. Endocr. **17**, 797 (1957).
ROSSIER, P. H.: Der Grundumsatz und seine Bedeutung. Helv. med. Acta **18**, 261 (1951).
TALBOT, N. B.: Basal metabolism standards for children. Amer. J. Dis. Child. **55**, 455 (1938).
TATA, J. R.: Basal metabolic rate and thyroid hormones. In: Advances in metabolic disorders I. New York: Academic Press 1964.
WEDGWOOD, R. J., BASS, D. E., KLIMAS, J. A., KLEEMAN, C. R., QUINN, M.: Relationship of body composition to basal metabolic rate in normal man. J. appl. Physiol. **6**, 317 (1955).

Ankle Reflex Time

BINSWANGER, J., STUDER, H., WYSS, F.: Der Ablauf der Sehnenreflexe bei Funktionsstörungen der Schilddrüse. Helv. med. Acta **28**, 482 (1961).
CANLORBE, P., CHEFUEUX, A.: Le réflexogramme achilléen chez l'enfant. Sem. Hôp. Paris **41**, 1253 (1965).
EMRICH, D., MAROUGIN, F.: Die Registrierung des Achillessehnenreflexes als diagnostisches Hilfsmittel bei Schilddrüsenerkrankungen. Dtsch. med. Wschr. **91**, 101 (1966).
FOWLER, P. B. S., BANIM, S. O., IKRAM HAMID: Prolonged ankle reflex in anorexia nervosa. Lancet **1972 II**, 307.
GORDON, M. B.: The Achilles Reflex Test (A.R.T.) in the diagnosis of thyroid dysfunction. Manhassel, N.Y.: Pierson 1962.
IMBACH, T.: Normalwerte der Achillessehnenreflexzeit beim Kind. Helv. paediat. Acta **24**, 463 (1969).
LAWSON, J. D.: The free Achilles reflex in hypothyroidism and hyperthyroidism. New Engl. J. Med. **259**, 761 (1958).
MILES, D. W., SURVEYOR, I.: Role of the ankle-jerk in the diagnosis and management of thyroid disease. Brit. med. J. **1965 I**, 158.
NUTTALL, F. Q., DOE, R. P.: The Achilles reflex in thyroid disorders. A critical evaluation. Ann. intern. Med. **61**, 269 (1964).
REINFRANK, R. F., KAUFMANN, R. P., WETSTONE, H. J., GLENNON, J. A.: Observations of the Achilles reflex test. J. Amer. med. Ass. **199**, 1 (1967).
RIVES, K. L., FURTH, E. D., BECKER, D. V.: Limitations of the ankle jerk test. Ann. intern. Med. **62**, 1139 (1965).
TSCHUDI, N.: Die Bedeutung des Achillessehnenreflexes in der Schilddrüsenfunktionsdiagnostik im Vergleich mit anderen Schilddrüsenfunktionsprüfungen. Praxis **31**, 858 (1966).
WEISS, S., FANKHAUSER, S.: Veränderungen der Achillessehnenreflexzeit bei Patienten mit Niereninsuffizienz. Helv. med. Acta, Suppl. **46**, 135 (1967).
ZACHMANN, M.: Influence of glucose and insulin administration on the Achilles tendon reflex time. Brit. med. J. **1967 II**, 528.

Plasma Cholesterol

ESCAMILLA, R.F.: Why is there no elevation of the plasma cholesterol level in pituitary myxedema? J. clin. Endocr. **13**, 746 (1953).

PETERS, J.P., MAN, E.B.: The significance of serum cholesterol in thyroid disease. J. clin. Invest. **29**, 1 (1950).

ROSENMANN, R.H., BYERS, S.O., FRIEDMAN, M.: The mechanism responsible for the altered blood cholesterol content in deranged thyroid states. J. clin. Endocr. **12**, 1287 (1952).

TSH Radioimmunoassay

BAKKE, J.L.: The assay of human thyrotropin by 21 different assay laboratories in 9 different countries using 14 different methods. In: Current topics in thyroid research. New York: Academic Press 1965.

Editorial: Thyrotrophin immunoassay. Brit. med. J. **1971 IV**, 761.

HERSHMAN, J., PITTMAN, J.A.: Utility of the radioimmunoassay of serum thyrotrophin in man. Ann. intern. Med. **74**, 481 (1971).

LEMARCHAND-BÉRAUD, TH., FELBER, J.P., VANNOTTI, A.: Développement d'une méthode radio-immunologique pour la détermination de la thyréostimuline. Résultats préliminaires. Schweiz. med. Wschr. **95**, 772 (1965).

MAYBERRY, W.E., GHARIB, H., BILSTAD, J.M., SIZEMORE, G.M.: Radioimmunoassay of human thyrotrophin. Ann. intern. Med. **74**, 471 (1971).

ODELL, W.D., WILBER, J.F., PAUL, W.E.: Radioimmunoassay of thyrotropin in human serum. J. clin. Endocr. **25**, 1178 (1965).

— — UTIGER, R.D.: Studies of thyrotropin physiology by means of radioimmunoassay. Recent Progr. Hormone Res. **23**, 47 (1967).

UTIGER, R.D.: Radioimmunoassay of human plasma thyrotropin. J. clin. Invest. **44**, 1277 (1965).

TRH Stimulation Test

ANDERSON, M.S., BOWERS, C.Y., KASTIN, A.J., SCHALCH, D.S., SCHALLY, A.V., SNYDER, P.J., UTIGER, R.D., WILBER, J.F., WISE, A.J.: Synthetic thyrotropin releasing hormone. A potent stimulator of thyrotropin secretion in man. New Engl. J. Med. **285**, 1279 (1971).

COSTOM, B.H., GRUMBACH, M.N., KAPLAN, S.L.: Effect of thyrotropin-releasing hormone on serum thyroid-stimulating hormone. An approach to distinguishing hypothalamic from pituitary forms of idiopathic hypopituitary dwarfism. J. clin. Invest. **50**, 2219 (1971).

FLEISCHER, N., BURGUS, R., VALE, W., DUNN, T., GUILLEMIN, R.: Preliminary observations on the effect of synthetic thyrotropin releasing factor on plasma thyrotropin levels in man. J. clin. Endocr. **31**, 109 (1970).

HAIGER, E.D., PITTMAN, J.A., HERSHMAN, J.M., BAUGH, C.M.: Direct evaluation of pituitary thyropin reserve utilizing synthetic thyrotropin releasing hormone. J. clin. Endocr. **33**, 573 (1971).

HERSHMAN, J.H., PITTMAN, J.A.: Response to synthetic thyrotropin-releasing hormone in man. J. clin. Endocr. **31**, 457 (1970).

ORMSTON, B.J., GARRY, R., CRYER, R.J., BESSER, G.M., HALL, R.: Thyrotropin-releasing hormone as a thyroid function test. Lancet **1971 a II**, 10.

— KILBORN, J.R., GARRY, R., AMOS, J., HALL, R.: Further observations on the effect of synthetic thyrotrophin-releasing hormone in man. Brit. med. J. **1971 b II**, 199.

STAUB, J.J., WEISS, S., KOHLER, H., BÜRGI, H., KOENIG, M.P., STUDER, H.: Effekt von peroral verabreichtem "TSH releasing hormone" (TRH) auf die Thyroxinkonzentration des Blutes. Schweiz. med. Wschr. **36**, 1295 (1971).

TSH Stimulation Test

BISHOPRIC, G.A., GARRETT, N.H., NICHOLSON, W.N.: Clinical value of the TSH stimulation test in the diagnosis of thyroid disease. Amer. J. Med. **18**, 15 (1955).

FORE, W., WYNN, J.: The thyrotropin stimulation test. Amer. J. Med. **40**, 90 (1966).

JEFFRIES, W.MC.K., LEVY, R.P., PALMER, W.G., STORAASLI, J.P., KELLY, L.W.: The value of a single injection of thyrotropin in the diagnosis of obscure hypothyroidism. New Engl. J. Med. **249**, 876 (1953).

SCHNEEBERG, N.G., PERLOFF, W.H., LEVY, L.M.: Diagnosis of equivocal hypothyroidism using TSH. J. clin. Endocr. **14**, 223 (1954).

SKANSE, B.: The use of thyrotropin in the differential diagnosis of primary and secondary hypothyroidism. Acta endocr. (Kbh.) **13**, 358 (1953).

TAUNTON, O.D., MCDANIEL, H.G., PITTMAN, J.A., JR.: Standardization of TSH testing. J. clin. Endocr. **25**, 266 (1965).

TSH Reserve Test with Carbimazole

BRICAIRE, H., JOLY, J., MATHIEUDE FOSSEY, B., COMPARET, J., BAILLET, J.: Exploration du couple hypophyso-thyroïdien à l'aide d'un dérivé de l'imidazol. Presse méd. **72**, 2505 (1964).

IFF, H.W., BURGER, A., STUDER, H., WYSS, F.: Thyrotrophin in rat and human serum after withdrawal of carbimazole. Amer. J. Physiol. **213**, 250 (1967).

JENSEN, S.E.: A new way of measuring thyrotropin (TSH) reserve. J. clin. Endocr. **29**, 409 (1969).

MORNEX, R., BERTHEZÈNE, F.: Comments on a proposed new way of measuring thyrotropin (TSH) reserve. J. clin. Endocr. **31**, 587 (1970).

POWELL, G.F., BLIZZARD, R.M.: An attempt to establish a test for thyrotropin (TSH) reserve. J. clin. Endocr. **26**, 1389 (1966).

SCHNEEBERG, N.G., KANSAL, P.C.: Commentary on a proposed TSH-reserve test. J. clin. Endocr. **26**, 579 (1966).

STUDER, H.: Der TSH-Reservetest mit Carbimazol. Helv. med. Acta **29**, 275 (1962).

— GREER, M.A.: Thyroid function during the rebound phase following the discontinuation of antithyroid drugs. Endocrinology **80**, 52 (1967).

— WYSS, F., IFF, H.W.: A TSH reserve test for detection of mild secondary hypothyroidism. J. clin. Endocr. **24**, 965 (1964).

Triiodothyronine Suppression Test

BURKE, G.: The triiodothyronine suppression test. Amer. J. med. **42**, 600 (1967).

FRIIS, T.: Effect of l-Triiodothyronine on the thyroid gland and its clinical application (triiodothyronine suppression test). Acta med. scand. **173**, 569 (1963).

KRISTENSEN, H.P.Ø., DYRBYE, M., CHRISTENSEN, L.K.: The triiodothyronin suppression test. Acta med. scand. **173**, 411 (1963).

WERNER, S.C., SPOONER, M.A.: A new and simple test for hyperthyroidism employing l-triiodothyronine and the 24 hour I^{131} uptake method. Bull. N.Y. Acad. Med. **31**, 137 (1955).

Perchlorate Discharge Test

BASCHIERI, L., BENEDETTI, G., LUCA, F. DE, NEGRI, M.: Evaluation and limitations of the perchlorate test in the study of thyroid function. J. clin. Endocr. **23**, 786 (1963).

STANLEY, M. M., ASTWOOD, E. B.: The accumulation of radioactive iodide by the thyroid gland and the effect of thiocyanate on its discharge. Endocrinology **42**, 107 (1948).

Test for Deiodinase Deficiency

ALBERT, A., KEATING, F. R., JR.: Metabolic studies with I^{131} labeled thyroid compounds: Distribution and excretion of radiodiiodotyrosine in human beings. J. clin. Endocr. **11**, 996 (1951).
GRAY, H. W., HOOPER, L. A., GREIG, W. R.: An evaluation of the twenty-minute perchlorate discharge test. J. clin. Endocr. **37**, 351 (1973).
— — — MCDOUGALL, I. R.: A twenty-minute perchlorate discharge test. J. clin. Endocr. **34**, 594 (1972).
REINWEIN, D., KLEIN, E.: Eine besondere Form von Jodfehlverwertung bei sporadischem Kretinismus. Schweiz. med. Wschr. **93**, 1213 (1963).
STANBURY, J. B., MEIJER, J. B. A., KASSENAR, A. A. H.: The metabolism of iodotyrosines. I. The fate of mono- and di-iodotyrosine in normal subjects and in patients with various diseases. J. clin. Endocr. **16**, 735 (1956).
— — — The metabolism of iodotyrosines. II. The metabolism of mono- and di-iodotyrosine in certain patients with familial goiter. J. clin. Endocr. **16**, 848 (1956).

Serum Antibodies to Thyroid Tissue

ANDERSON, J. W., MCCONAHEY, W. M., ALARCON-SEGOVIA, D., EMSLANDER, R. F., WAKIM, K. G.: Diagnostic value of thyroid antibodies. J. clin. Endocr. **27**, 937 (1967).
BUCHANAN, W. W., KOUTRAS, D. A., CROOKS, J., ALEXANDER, W. D., BRASS, W., ANDERSON, J. R., GOUDIE, R. B., GRAY, K. G.: The clinical significance of the complement-fixation test in thyrotoxicosis. J. Endocr. **24**, 115 (1962).
BURGER, N.: Schilddrüsen-Autoantikörper. Praxis **53**, 1646 (1964).
FORBES, I. J., ROITT, I. M., DONIACH, D., SOLOMON, T. L.: The thyroid cytotoxic autoantibody. J. clin. Invest. **41**, 996 (1962).
HJORT, T.: The occurrence of antibody against "second colloid antigen" (CA-2 antibody) in patients with and without thyroid disease. Acta med. scand. **174**, 147 (1963).
ROITT, I. M., DONIACH, D.: Thyroid auto-immunity. Brit. med. Bull. **16**, 152 (1960).

Thyroid Biopsy

GALVAN, G., POHL, G. B.: Feinnadelpunktion und zytologische Auswertung von 2523 kalten Strumaknoten. Dtsch. med. Wschr. **98**, 2107 (1973).
HAMLIN, E., VICKERY, A. L.: Needle biopsy of the thyroid gland. New Engl. J. Med. **254**, 742 (1956).
HAWK, W. A., CRILL, G., HAZARD, J. B., BARRETT, D. L.: Needle biopsy of the thyroid gland. Surg. Gynec. Obstet. **122**, 1053 (1966).

Clinical Indices. Value of Laboratory Tests

BILLEWICZ, W. Z., CHAPMAN, R. S., CROOKS, J., DAY, M. E., GOSSAGE, J., WAYNE, E., YOUNG, J. A.: Statistical methods applied to the diagnosis of thyroid disease. Quart. J. Med. **38**, 255 (1969).
CHEW, C. K., PAIN, R. W.: A comparison of thyroid function tests. Med. J. Aust. **1962 I**, 435.
CROOKS, J., MURRAY, I. P. C., WAYNE, E. J.: Statistical methods applied to the diagnosis of thyrotoxicosis. Quart. J. Med. **28**, 211 (1959).
FRAGU, P., THOUIN, A., BAZIN, J. P., TUBIANA, M.: Comparaison de la valeur diagnostique de différents tests fonctionnels thyroïdiens. Intérêt de l'utilisation des fonctions discriminantes. Ann. Endocr. (Paris) **33**, 5 (1972).
GORDON, A., GROVER, N. B., BARZILAI, D., EHRENFELD, E. N., LEWITUS, Z., GROSS, J.: Computer study of thyroid disease. II. Results. Israel J. med. Sci. **6**, 333 (1970).
GURNEY, C., OWEN, S. G., HALL, R., ROTH, M., HARPER, M., SMART, G. A.: Newcastle thyrotoxicosis index. Lancet **1970 II**, 1275.
HARVEY, R. F.: Indices of thyroid function in thyrotoxicosis. Lancet **1971 II**, 230.
LUDDECKE, H. F.: Basal metabolic rate, protein-bound iodine and radioactive iodine uptake: a comparative study. Ann. intern. Med. **49**, 305 (1958).
RIVES, K. L., FURTH, E. D., BECKER, D. V.: Limitations of the ankle jerk test. Intercomparison with other tests of thyroid function. Ann. intern. Med. **62**, 1139 (1965).

Added in Proofs:

DEGROOT, L. J., PALOYAN, E.: Thyroid carcinoma and radiation. J. Americ. med. Ass. **225**, 487 (1973).
GATTERAU, A., BÉNARD, B., BELLABARBA, D., VERDY, M., BRUN, B.: Congenital goiter in four euthyroid siblings with glandular and circulating iodoproteins and defective iodothyronine synthesis. J. clin. Endocr. **37**, 118 (1973).
KUSAKABE, T.: A goitrous subject with defective synthesis of diiodotyrosine due to thyroglobulin abnormalities. J. clin. Endocr. **37**, 317 (1973).
LARSEN, P. R.: Triiodothyronine: Review of recent studies of its physiology and pathophysiology in man. Metabolism **21**, 1073 (1972).
NEINAS, F. W., GORMAN, C. A., DEVINE, K. D., WOOLNER, L. B.: Lingual thyroid. Ann. intern. Med. **79**, 205 (1973).
OGIHARA, T., YAMAMOTO, T., AZUKIZAWA, M., MIYAI, K., KUMAHARA, Y.: Serum thyrotropin and thyroid hormones in the course of subacute thyroiditis. J. clin. Endocr. **37**, 602 (1973).
POMMIER, J., DEME, D., NUNEZ, J.: Effect of iodide concentration on thyroxine synthesis catalyzed by thyroid peroxidase. Europ. J. Biochem. **37**, 406 (1973).
RILEY, F. C.: Orbital pathology in Graves' disease. Mayo Clin. Proc. **47**, 975 (1972).
RUBENSTEIN, H. A., BUTLER, V. P., WERNER, S. C.: Progressive decrease in serum triiodothyronine concentrations with human aging: radioimmunoassay following extraction of serum. J. clin. Endocr. **37**, 247 (1973).
RUSKIN, A. V., TANG, S. C., SHENKMAN, L., MITSUMA, T., HOLLANDER, C. S.: Serum triiodothyronine concentrations in infancy, childhood, adolescence and pediatric disorders. J. clin. Endocr. **37**, 235 (1973).
THILLY, C. H., DELANGE, F., GOLSTEIN-GOLAIRE, J., ERMANS, A. M.: Endemic goiter prevention by iodized oil: a reassessment. J. clin. Endocr. **36**, 1196 (1973).

VII. Adrenal Cortex

A. LABHART

With Contributions by

CHR. HEDINGER, G. KISTLER, J. MÜLLER, A. PRADER, R. SIEBENMANN, G. TÖNDURY, and M. ZACHMANN

A. Historical Dates

1563 BARTHOLOMAEUS EUSTACHIUS SANCTOSEVERINATUS describes the adrenals and recognizes them as an organ in his treatise "De glandulis quae renibus incumbunt".

1855 THOMAS ADDISON gives the classic description of adrenal insufficiency and of the disease which bears his name in his book "On the constitutional and local effects of disease of the suprarenal capsules".

1856 BROWN-SÉQUARD discovers in animal experiments that the adrenals are necessary to life.

1896 SIR WILLIAM OSLER recognizes the efficiency of perorally administered adrenal extracts in ADDISON'S disease.

1926 SMITH demonstrates that hypophysectomy leads to atrophy of the adrenals and EVANS succeeds in preventing this atrophy by the administration of pituitary extracts.

1928–1930 effective adrenal extracts are made by ROGOFF and STEWART, HARTMANN and MCARTHUR, PFIFFNER and SWINGLE.

1932 CUSHING describes the syndrome of pituitary-adrenal hyperactivity.

1933 LOEB discovers disturbances of serum electrolytes in ADDISON'S disease and bases the treatment with sodium chloride on these discoveries.

1937–1952 Isolation, elucidation of the constitution, and synthesis of the adrenal hormones corticosterone, deoxycorticosterone, cortisone and cortisol by REICHSTEIN, KENDALL, WINTERSTEINER and collaborators.

1942 LI and SAYERS isolate ACTH.

1946 SELYE describes the general adaptation syndrome.

1948 HENCH and collaborators detect the anti-inflammatory effect of cortisone.

1953–1955 Isolation and elucidation of the constitution of aldosterone by SIMPSON and TAIT, WETTSTEIN and NEHER, REICHSTEIN and VON EUW. Synthesis by WETTSTEIN and SCHMIDLIN.

1954 CONN describes primary aldosteronism.

1958 GROSS suggests the control of aldosterone secretion by angiotensin.

1960 DAVIS and GENEST confirm the regulation of aldosterone secretion by angiotensin II.

1962 HOFMANN, LI, and SCHWYZER describe the amino-acid sequence of ACTH.

1966 SCHWYZER and SIEBER succeed in synthesizing beta-corticotropin.

B. Embryology, Gross Anatomy and Histology

G. TÖNDURY and G. KISTLER

Embryology. The two main components of the adrenal (suprarenal) gland, the *cortex* and the *medulla*, differ not only in morphology and function, but also in origin. The cortex develops from cells of the coelomic epithelium and is therefore of mesodermal origin, while the chromaffin and sympathetic ganglion cells of the medulla are derived from the neural ectoderm. In amphibians, reptiles, and birds the chromaffin cells are diffusely distributed within the cortical tissue throughout life. In mammals, however, the cortex finally surrounds the medulla like a capsule.

In the four-week-old human embryo, coelomic epithelial (mesothelial) cells situated on both sides between the root of the mesentery and the gonadal anlagen begin to proliferate and to invade the underlying mesenchymal tissue. Here, they differentiate into compact, acidophilic epithelial masses which lose contact with the coelomic epithelium and form the *primitive or fetal cortex* of the suprarenal glands. During the 6th embryonic week, neural ectodermal elements, the pheochromoblasts, start to invade the primitive cortex (see Chap. VIII, p. 419). Soon afterward, they can be observed singly or in small groups between the cortical cells. Early in the third month, the coelomic epithelium proliferates again and a

new wave of mesothelial cells is shed into the underlying mesenchyma. These cells, which are smaller than those of the first wave, surround the fetal cortex to form the *definitive cortex* of the adrenal gland. During the last month of intra-uterine life, the fetal cortex begins to regress. At the time of birth, the suprarenal glands are nevertheless very large, their volume being approximately one third that of the kidneys. In the newborn, the atrophy of the fetal cortex proceeds rapidly, except for its outermost cell layers which appear to be taken up into the zona reticularis of the definitive cortex (see below). In the adult human, the size of the suprarenal glands is only about 1/30 of that of the kidneys.

The function of the primitive suprarenal cortex is still poorly understood. Since the adrenal glands are atrophic in anencephalic infants (in whom the hypophysis is absent), it is assumed that the very large size of the fetal glands is due to the release of adrenocorticotrophic hormone (ACTH) from the fetal pituitary. ACTH secretion by the fetus can also be inhibited by cortisone administration to the mother. In such cases, the fetal suprarenal gland is often found to be hypo- or even atrophic.

Gross Anatomy. In the adult, each of the adrenal glands weighs about 3–5 g. They are situated retro-peritoneally and on the supero-medial aspect of the front of the kidneys. Both glands are surrounded by adipose tissue and by the renal fasciae, to which they adhere firmly. Unlike the kidneys, the glands are not displaced during respiration or changes in posture. The *right* adrenal gland is pyramidal in shape. Its base rests on the kidney and its medial portion projects to some extent behind the inferior vena cava. Behind, it lies against the diaphragm, whereas in front, it is in direct contact with the right lobe of the liver, with the dorsal wall of the vena cava and with the peritoneum. The *left* suprarenal gland is usually semilunar in shape and more flattened. In front, it is in close contact with the splenic artery, with the peritoneum of the lesser sac and with the pancreas. Its dorsal portions rest on the diaphragm.

The phrenic artery supplies the adrenal gland with multiple branches, the *superior* adrenal arteries. The *middle* suprarenal artery reaches the gland directly from the aorta, whereas the *inferior* adrenal arteries emerge from the renal artery. The arrangement of these blood vessels varies widely not only in different people, but also on the two sides of the body. The suprarenal *vein* leaves the gland in the hilus. In addition, some small veins accompany the arteries. The left adrenal vein enters the renal vein, whereas the right vein supplies the inferior vena cava. A number of lymphatic vessels arise in the adrenal medulla. They follow the veins and supply adjacent lymph nodes. In contrast, the cortex contains only a very few lymphatics.

The predominantly *preganglionic, sympathetic nerve fibers* of the adrenal glands arise in the celiac plexus or are branches of the thoracic and lumbar splanchnic nerves. Most of these fibers supply the specific medullary cells; a few of them, however, contact the ganglion cells in the organ capsule and in the medulla. Postganglionic nerve fibers extend to the blood vessels walls. No parasympathetic fibers seem to enter the glands.

Histology. A thick capsule of collagenous connective tissue surrounds the suprarenal gland and extends as trabeculae into the cortex. It contains an extensive arterial plexus which is supplied by the various suprarenal arteries (see above). The cortical arterioles arising from this plexus branch into the sinusoidal capillaries which surround the cords and groups of cortical parenchymal cells. In addition, the capsule and its trabeculae display some lymphatic vessels as well as a nerve plexus. The latter includes a large number of mainly preganglionic fibers and a few sympathetic ganglion cells. Its branches penetrate the cortex in the trabeculae and terminate in the medulla (see Chap. VIII, p. 420). A framework of reticular fibers extends between the trabeculae, the organ capsule and the blood vessels. It supports the specific cortical cells as well as the medullary cells, and surrounds the capillary network of the organ.

The *adrenal cortex* constitutes approximately 80% of the total organ volume and is composed of three easily distinguishable concentric zones. The thin *zona glomerulosa* adjacent to the organ capsule consists of groups of rather small, epitheloid cells which contain a deeply staining nucleus displaying one or two nucleoli. The cytoplasm is acidophilic and contains some small clumps of basophilic material. The small lipid droplets and the elongated mitochondria are randomly distributed throughout the cytoplasmic matrix, whereas the Golgi apparatus is situated in a juxtanuclear position. Both reticular fibers and sinusoidal capillaries surround the cell groups. Electron-microscopically, the zona glomerulosa cells are characterized by an anastomosing network of smooth-surfaced endoplasmic reticulum extending all over the cell body. Only a few profiles of granular endoplasmic reticulum can usually be observed. The mitochondria possess lamellar cristae thus differing from those of the other cortical zones.

The cytoplasmic matrix contains large numbers of polyribosomes and the lipid droplets are often arranged in small groups. The Golgi apparatus does not differ from that of the cells of other organs.

The cell columns of the largest cortical zone, the *zona fasciculata*, are continuous with the cell groups of the zona glomerulosa. Their polygonal cells form long cords which are usually one to two cells thick and are arranged radially to the organ capsule. The individual columns are separated by reticular fibers and by the sinusoidal capillaries. The fasciculata cells often contain two nuclei, and their cytoplasm is characterized by the presence of large numbers of lipid droplets. In routine histological sections, the cytoplasm appears highly vacuolated. When observed under the electron microscope, the cells of this zone display an extensive network of agranular endoplasmic reticulum. A few parallel arrays of granular endoplasmic reticulum are usually situated in the neighborhood of the nucleus (or nuclei). The rounded mitochondria vary considerably in size but are usually much larger than those of the other cortical zones. Their numerous cristae appear as vesicular invaginations of the inner mitochondrial membrane or as vesicles lying free in the mitochondrial matrix. The lipid droplets are dispersed throughout the cytoplasmic matrix. In the Golgi region, which is generally larger than that of the other zones, lysosomes as well as deposits of lipofuscin pigment are often present.

The *zona reticularis* adjacent to the adrenal medulla consists of an anastomosing network of epitheloid cells which vary considerably in size and shape. Within the cell groups, "light" and "dark" cells can be distinguished, the latter presumably being degenerative elements, since they contain hyperchromatic and shrunken nuclei and large masses of lipofuscin pigment. Compared with the fasciculata cells, the light cells of the zona reticularis contain a much smaller number of lipid droplets. The shape of the mitochondria and the amount of agranular endoplasmic reticulum, however, do not differ significantly.

The surface of the cortical cells of the three zones is usually enlarged by small infoldings as well as by numerous small microvilli which project into the intercellular and perivascular spaces. The endothelium of the sinusoidal capillaries is, over large regions, extremely attenuated and fenestrated. The endothelial fenestrae or pores are closed only by a very thin diaphragm which separates the blood from the basal lamina surrounding the sinusoids. A few small bundles of collagen fibrils are dispersed in the perivascular spaces. They correspond to the reticular fibers revealed by light microscopy.

C. Biochemistry

J. MÜLLER

1. Steroid Hormones

The hormones of the adrenal cortex, the gonads and the placenta, provitamin D, cholesterol, bile salts, and the cardiac glycosides are steroids, i.e. structural derivatives of *sterane*, a completely hydrated cyclopentanophenanthrene. Sterane consists of three hexagonal rings and one pentagonal ring, which are designated by the letters A, B, C, and D. Each carbon atom is marked by a number (Fig. 1).

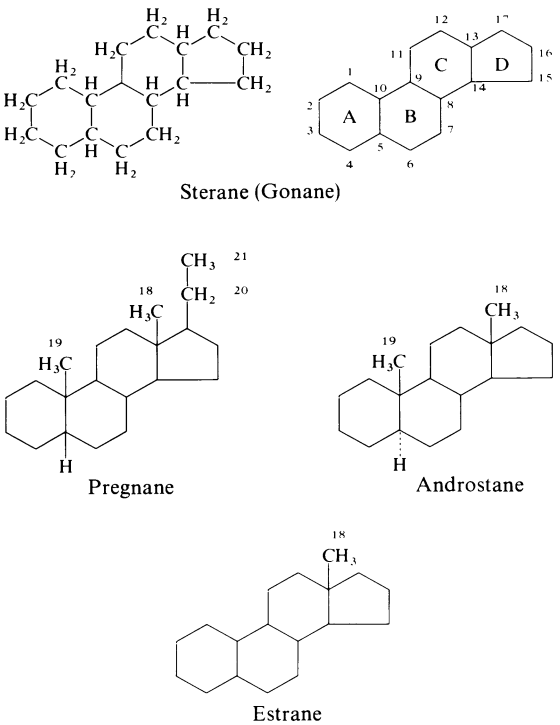


Fig. 1. Basic structures of steroid hormones

The steroid structure can be expanded by a side chain on C-17 and by angular methyl groups on C-10 and C-13. The individual steroid hormones are further characterized by double bonds, hydroxyl, and ketone groups.

The hormones of the adrenal cortex and the corpus luteum have 21 C-atoms and are derived

from *pregnane*, a sterane with two angular methyl groups and an ethyl side chain. The male sexual hormones are derived from *androstane* with 19 C-atoms, the female sexual hormones from *estrane* with 18 C-atoms.

The additional carbon atoms are numbered in a continuous sequence (angular methyl groups: C-18, C-19, ethyl side chain: C-20, C-21).

2. Stereoisomerism and Nomenclature*

Different stereoisomerisms of steroids are due to a number of asymmetric carbon atoms. In general, the stereo structure of a steroid is clearly defined by the nomenclature, because the biological activity is closely related to it. Thus, etiocholanolone, the stereoisomer of the active androgen androsterone, has no androgenic activity but is an active pyrogenic substance (Fig. 5, Chap. IX, p. 448).

Sterane has six asymmetrical carbon atoms. Thus, $2^6 = 64$ stereoisomerisms are theoretically possible. However, in natural steroid hormones rings B/C and C/D are always connected by trans-fusion; the H-atoms or methyl groups on the two angular C atoms are placed on opposite faces of the molecular plane. The rings A and B are combined either by cis- or by trans-fusion. Additional stereoisomerisms are due to side chains, methyl and hydroxyl groups. The methyl group on C-10 has been chosen as the stereo point of reference for the position of such substitutes. It is considered to lie on the near side of the plane of writing and is designated by a solid valency stroke and the affix β. In contrast, substitutes lying on the far side of the plane of writing are designated by dotted valency strokes and the affix α. In natural steroids, the side chain at C-17 is always in the β-position. In a C_{21} steroid, a 17-hydroxyl group is therefore always in the α-position, whereas it can occur in the α- or β-position in a C_{18} or C_{19} steroid. In adrenal cortical steroids, an 11-hydroxyl group is always in the β-position, but 3- or 20-hydroxyl groups can occur in α- or β-position.

Steroids hydroxylated in certain positions can be *conjugated* with sulfuric acid or glucuronic acid. Steroid conjugates are generally inactive. They are always more water-soluble, and usually more rapidly excreted in the urine than unconjugated steroids. For a long time they were considered as breakdown and waste products of steroid hormones. Recently, however, the question of the physiologic role of steroid conjugates was reexamined, since it was found that steroid sulfates were secreted by the adrenal glands into the blood stream and that conjugated androgens were biochemically transformed before being excreted in the urine (BAULIEU, 1965). Conjugation with sulfuric acid is an esterification, whereas conjugation with glucuronic acid is β-glycosidic.

In the chemical nomenclature, one differentiates between so-called *trivial names*, short and convenient designations for individual compounds, and *systematic* names, which are inconvenient but define the exact structure. They are formed by adding pre- and suffixes to the name of the basic carbon structure (Fig. 2) and define the type, number and position of all substitutes. Hydroxyl groups are referred to by the suffix "-ol" or the prefix "hydroxy-", ketone groups by the suffix "-one" or the prefix "keto-" (also "oxo-"). Double bonds are designated by the suffix "-ene"; the localization is given by inserting the number of the first of the two C-atoms involved. When a double bond connects two C-atoms with non-consecutive numbers, both numbers are written. Occasionally, the position of a double bond is denoted by a "Δ" prefix according to an outdated nomenclature system. The position of substitutes on optically active C-atoms are designated by α, β or ξ (unknown). The prefix "allo-" refers to trans-fusion of the A and B rings. The prefixes "desoxy-", "dehydro-", "dihydro-", "tetrahydro-", "epi-" and "iso-" are only used for trivial names. The trivial name of the most important adrenocortical hormone is *cortisol*, synonymous with *hydrocortisone*; its systematic name is

4-pregnene-11β,17α,21-triol-3,20-dione .

Terms such as "*corticoids*", "*corticosteroids*", and "*17-ketosteroids*", are frequently used to describe certain groups of steroids. Corticoids or corticosteroids refer to adrenal cortical hormones with 21 C-atoms which influence electrolyte and carbohydrate metabolism as well as their inactive breakdown products with an intact side chain. The group of 17-ketosteroids includes both the androgenically active and the hormonally inactive C-19 steroids with a ketone group in the 17-position.

Basic information about the structure and nomenclature of the steroid hormones for the non-chemist can be found in the reviews by ZIMMERMANN, KLYNE, MASON, HÜBENER, and STAIB. More detailed information is given by FIESER and FIESER.

* The nomenclature of the steroids is ruled by the recommendations of the International Union of Pure and Applied Chemistry (IUPAC-IUB 1967 Revised Tentative Rules for Steroid Nomenclature. Biochim. Biophys. Acta 1964, 453 (1968)).

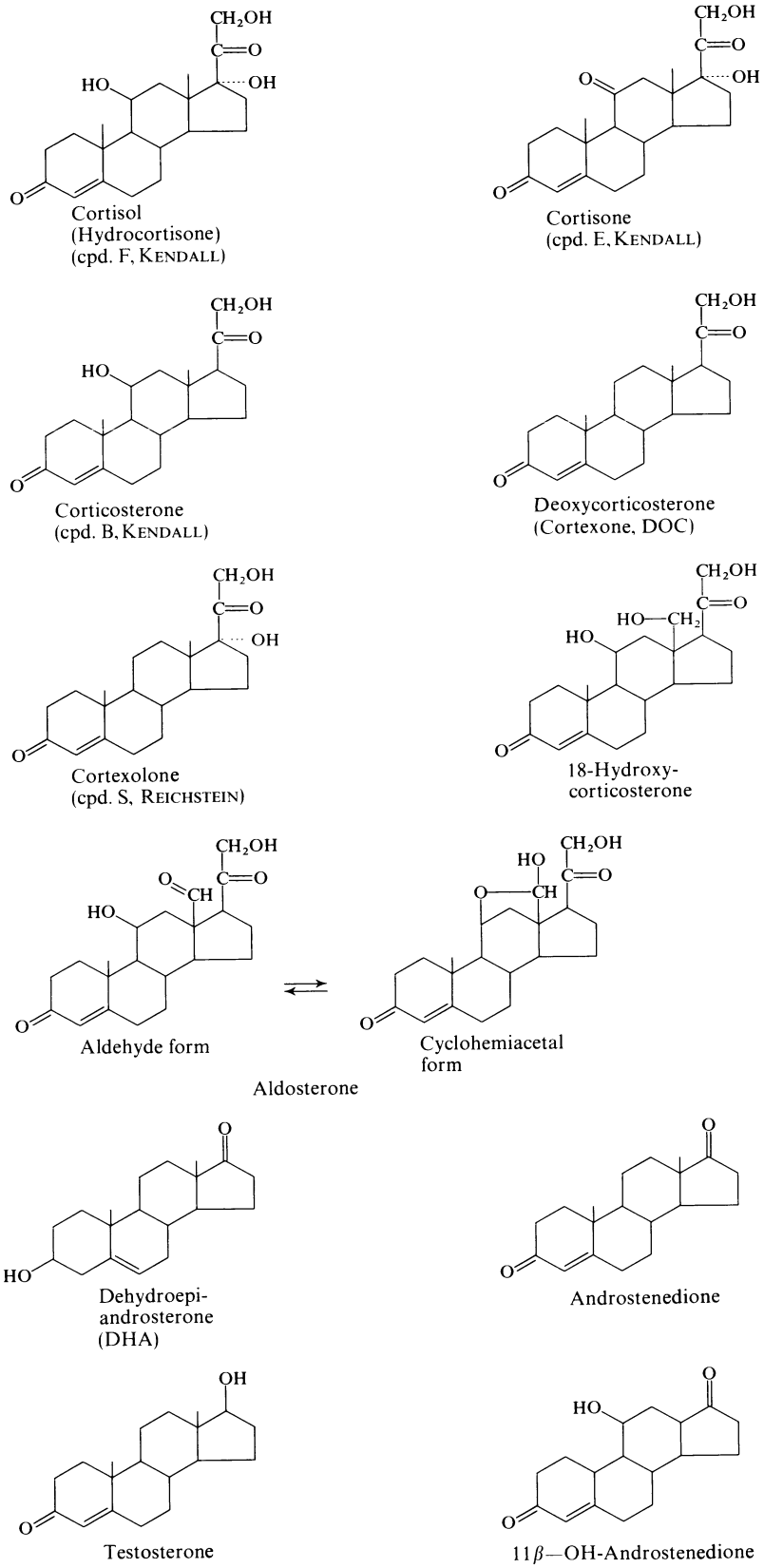


Fig. 2. The most important steroids of the adrenal cortex

3. The Adrenal Cortical Hormones

Among the approximately 70 different steroids which have been isolated from animal adrenal glands, cortisol, corticosterone, and androgens are the main secretory products of the human adrenal cortex. In addition, the mineralocorticoid aldosterone, secreted in small amounts, is of very great physiological importance. Along with these biologically active adrenal cortical hormones, other less active or inactive precursors or byproducts are also found in human adrenal venous blood, such as deoxycorticosterone (cortexone), cortexolone (compound S), 18-hydroxycorticosterone, 17α-hydroxyprogesterone, and 17α-hydroxypregnenolone. The androgen dehydroepiandrosterone is mainly secreted in the conjugated form as a sulfate. It was recently confirmed that the human adrenals secrete testosterone. However, there is as yet no direct evidence for production of estrogens by the human adrenal cortex. Measurable amounts of estrogens have been found in the urine of ovariectomized women, which increased in response to ACTH treatment and disappeared after adrenalectomy.

The concentrations of steroids in adrenal venous blood taken during operations under general anesthesia give only very limited information about normal secretion rates, because the adrenal glands are maximally stimulated by ACTH under these conditions. However, the basal daily secretion rates of the most important adrenocortical hormones have been indirectly determined by isotope dilution methods (see Table 1).

The ratio of cortisol to corticosterone secretion varies among the different species, but also among individuals within the same species. In man, cortisol predominates by a factor of 10:1, whereas in the rat corticosterone is the prevailing glucocorticosteroid.

For a review dealing with the amounts of steroids in adrenal venous blood in man and different animal species see YATES (1962).

4. The Biosynthesis of the Adrenal Cortical Hormones

The biosynthesis of the steroid hormones of the adrenal cortex have been studied most with the following experimental models:

1. Perfusion of adrenal glands *in vitro* or *in vivo*.
2. Incubation of slices of surviving adrenal tissue.
3. Incubation of adrenal homogenates or cell fractions.

These methods are very useful for isotope studies. Biological precursors (acetate, mevalonic acid, steroids) labeled with ^{14}C or tritium are added to the perfusion fluid or the incubation medium. The conversion to radioactive steroids is determined by extraction, isolation, and isotope counting.

The biosynthetic pathway of adrenal cortical hormones from acetyl coenzyme A via cholesterol, pregnenolone, and 17α-hydroxypregnenolone is outlined in Fig. 3. One-step conversions are designated by solid arrows, multistep conversions by dotted arrows. They are catalyzed by specific enzymes (desmolase, isomerase, 3β- and 18-dehydrogenase, 20α-, 22ξ-, 17α-, 21-, 11β-, 18-hydroxylases) and are dependent on coenzymes, such as NADH and NADPH. In the adrenal cortex as well as in other organs, cholesterol can be fully synthesized via the important intermediary products mevalonic acid and squalene. The biosynthetic pathway leading to cholesterol has been mainly elucidated by the investigations of BLOCH. Cholesterol is abundantly stored in the adrenal cortex, but can be only partially utilized for the production of adrenal cortical hormones.

Table 1. Adrenal cortical hormones of man

	Secretion rate (per day)	Concentration in peripheral plasma
Cortisol	15–40 mg	6 –25 µg/100 ml
Corticosterone	1– 4 mg	0.4 – 2.0 µg/100 ml
Aldosterone	50– 250 µg	2 –15 ng/100 ml
Cortexolone (compound S)	100–1500 µg	0.01– 0.11 µg/100 ml
Deoxycorticosterone (cortexone, DOC)	200– 800 µg	2 –10 ng/100 ml
18-Hydroxycorticosterone	150– 450 µg	
17α-Hydroxyprogesterone		
17α-Hydroxypregnenolone		
Dehydroepiandrosterone(-sulfate)	6–9 mg	
Androstenedione		
11β-Hydroxyandrostenedione		
Testosterone		
Estrogens (?)		

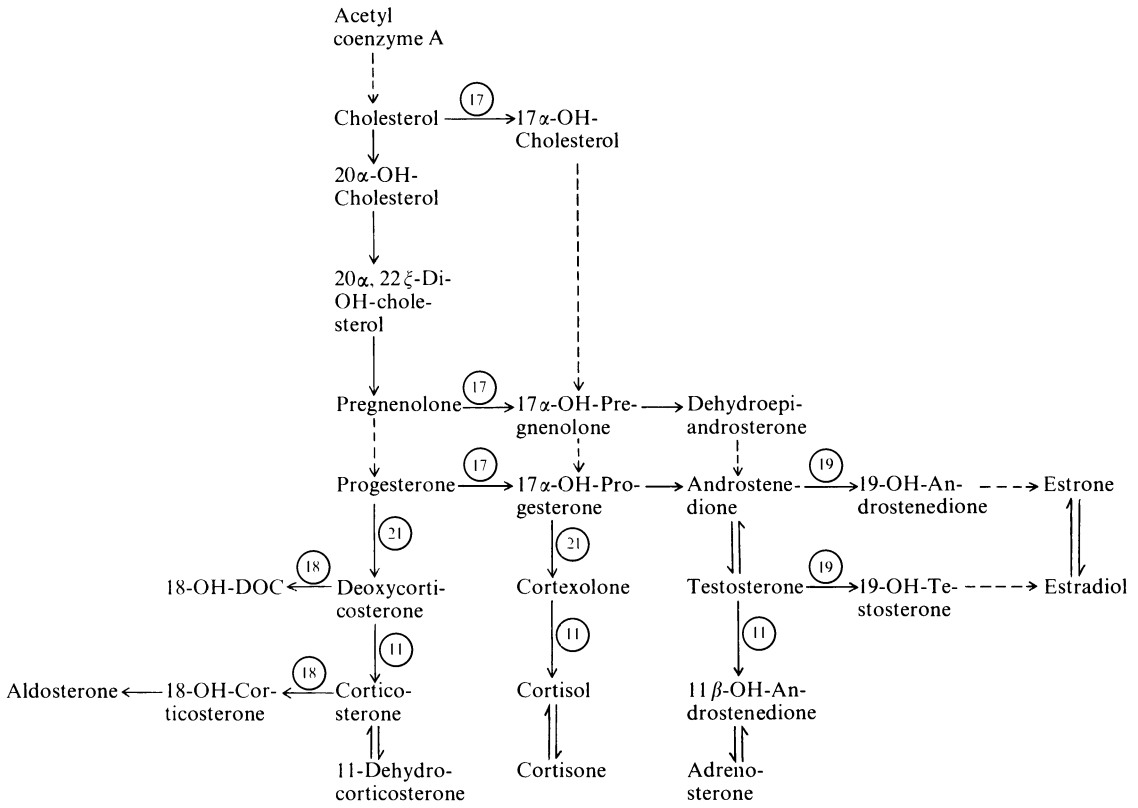


Fig. 3. The biosynthesis of adrenal cortical hormones. (Solid arrows: one-step conversions; broken arrows: multistep conversions. The circled numbers refer to hydroxylases)

Cholesterol is probably the normal precursor of all adrenal cortical hormones. In animals fed with radioactive cholesterol for several weeks the corticosteroids in the peripheral blood had the same specific radioactivity as the adrenal cholesterol and the serum cholesterol even during short-term stimulation with ACTH (WERBIN, 1961; KRUM, 1964).

Addition of ACTH to the perfusion fluid or the incubation medium of adrenal slices increases the steroid biosynthesis *in vitro* by a factor of 5–10, which corresponds to the stimulation of corticosteroid excretion in human urine during ACTH treatment. See p. 296 for the site and mode of action of ACTH.

Aldosterone is formed only in the zona glomerulosa, whereas all other steroid hormones are formed in the inner zones of the adrenal cortex. It is the only steroid with an aldehyde group in position 18. This group is probably formed in two stages, i.e. hydroxylation followed by dehydrogenation. The immediate precursor of aldosterone is most probably 18-hydroxycorticosterone. Aldosterone can occur as either a dihydroxyaldehyde or a cyclohemiacetal (Fig. 2). *In vivo*, however, it probably exists only in the latter form. The physiological significance of 18-hydroxycorticosterone and 18-hydroxy-DOC is unknown, apart from their possible role as precursors of aldosterone. 18-Hydroxy-DOC has a weak mineralocorticoid activity. In the human, the secretion rate of 18-hydroxycorticosterone is twice that of aldosterone. In the rat, 18-hydroxy-DOC is produced in very high amounts, but mainly by the zona fasciculata.

17α-Hydroxypregnenolone or 17α-hydroxyprogesterone appear to be essential intermediates in the biosynthesis of the androgens. A direct conversion of cholesterol to C_{19} steroids has never been demonstrated, though cholesterol sulfate can be converted to pregnenolone sulfate and this to dehydroepiandrosterone sulfate. If estrogens are really produced in the adrenals, androstenedione, testosterone and their 19-hydroxy derivatives are the most probable intermediates, as they are in the ovary and the placenta.

5. Transport of Adreno-Cortical Hormones

At normal concentrations, only a small part of the total blood cortisol and corticosterone is in the free form, i.e. susceptible to dialyzation and filtration.

Most of the 25 percent adsorbed onto the erythrocytes can be separated by washing with physiological saline solution. The uptake by leukocytes is of no quantitative importance.

At body temperature, 90 percent of the plasma cortisol is bound to an alpha-globulin. This protein is called "transcortin" or "corticosteroid-binding globulin" (CBG). It is a glycoprotein with a molecular weight of 52000. Transcortin has a high affinity to cortisol and corticosterone, but its binding capacity is limited. It is fully saturated at a plasma cortisol concentration of 20 µg/100 ml. Up to this concentration, only 10% of the plasma cortisol is in the free form. At higher concentrations, free plasma cortisol increases to 20–30%; the remaining 70–80% are then bound to albumin, which has a low affinity but a large capacity (Fig. 4).

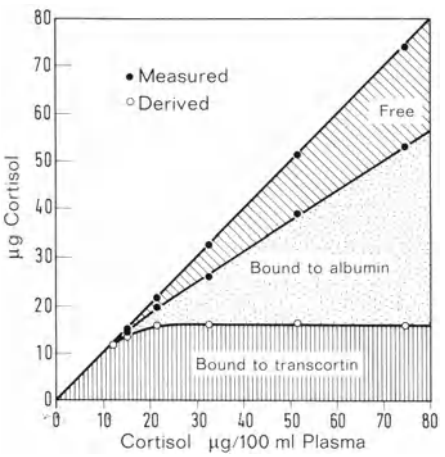


Fig. 4. Protein-binding of cortisol in normal human plasma at 4° C. (From W. H. DAUGHADAY and I. K. MARIZ, 1960)

The binding affinity to transcortin is determined by specific groups of the cortisol molecule. In addition to cortisol and corticosterone, deoxycorticosterone is also bound. On the other hand, transcortin-binding affinity to cortisol metabolites is very low, and aldosterone is bound only to other plasma proteins.

The concentration and binding capacity of transcortin are independent of ACTH. ACTH stimulation of the adrenal cortex induces a relatively larger increase in the free than in the bound plasma cortisol. The transcortin concentration is unaltered in adrenal insufficiency and in Cushing's syndrome, while a striking increase in transcortin level can be induced by estrogens. The amounts of estrogens produced by the ovaries during normal cycles are not sufficient for this effect, but transcortin levels are elevated in response to the high amounts of endogenous estrogens produced during pregnancy or to pharmacological doses of exogenous estrogens (oral contraceptive medication). This increase in plasma transcortin is always coincident with an increase in the plasma cortisol concentration and can at least partially explain why the plasma concentration of corticoids can be up to 3 times higher than normal during the last trimester of pregnancy with no obvious signs of hyperadrenocorticoidism. Conversely, transcortin can be decreased in liver diseases and dysproteinemia, thus accounting for the absence of adrenal insufficiency in spite of very low concentrations of plasma corticosteroids.

Transcortin concentration is much lower in the lymph than in the plasma. It is also very low in the cerebrospinal fluid. Correspondingly, the cortisol content in these two body fluids is lower than in the plasma. The transcortin-bound cortisol is very probably biologically inactive. In the extravascular space there is probably a state of equilibrium between the binding of cortisol to transcortin on the one hand, and to certain cell structures (hormone receptors) on the other hand. The cortisol bound to transcortin is also more slowly inactivated by liver enzymes *in vitro*. This is probably due to competition between binding sites of the transcortin and of the microsomes of the liver cells. Only the free plasma cortisol is filtered by the kidneys; a major part of it is reabsorbed by the tubules. The renal clearance of the free plasma cortisol is 40–50 ml per minute. When renal function is normal, the daily excretion of free cortisol in the urine is a good index of the concentration of the free plasma cortisol.

The physiological role of transcortin probably lies in a sort of buffering effect, which prevents rapid variations of the plasma cortisol level. Transcortin restrains the active cortisol from reaching the target organ and also protects it from rapid inactivation by the liver and excretion through the kidneys.

The plasma aldosterone is predominantly bound to albumin, and also to another protein which is probably not identical to transcortin. The relatively low degree of protein binding of plasma aldosterone could provide a partial explanation for the very low plasma concentration and the short biological half-life of this hormone.

6. Metabolic Breakdown and Excretion of Adrenal Cortical Hormones

In the organism, cortisol is very rapidly converted to inactive steroids by enzymes. Only very small amounts (less than 1%) are excreted

unaltered in the urine. The liver plays the most important part in the inactivation of the steroid hormones. Catabolism by other organs is not quantitatively important. The major reactions of cortisol metabolism usually occur in the following order:

1. Reduction of the double bond between C-4 and C-5.
2. Reduction of the 3-keto group.
3. Reduction of the 20-keto group.
4. Fission of the side chain at C-17.

Most of the cortisol is metabolized to stage 2 (40–50%) or stage 3 (20–30%). Less than 10% is converted to 17-ketosteroids.

Cortisol can be reversibly converted to cortisone in the liver under the action of an 11β-dehydrogenase.

Dihydrocortisone, dihydrocortisol, and their allo-isomers are formed by the action of 5α- and β-reductases. The reduction of the double bond is practically irreversible and is probably rate-limiting for subsequent metabolic breakdown. NADPH is an essential coenzyme. The reaction is also dependent on thyroxine. It is accelerated in hyperthyroidism and delayed in hypothyroidism and in liver diseases.

The second reaction leads to the formation of tetrahydro derivatives by reduction of the 3-keto group. This reaction is reversible. NADPH and NADH are equi-effective coenzymes.

A part of these tetrahydro derivatives is further converted to cortol and cortolone by reduction of the 20-ketone group. Finally, a small percentage is broken down to 11β-hydroxy-17-ketosteroids and 11-17-diketosteroids by side-chain fission.

Inactivation through 6β-hydroxylation occurs mainly in the newborn and only to a small extent in adults.

The action of the enzyme glucuronyl-transferase conjugates the reduced metabolites to uridine-diphosphate glucuronic acid to form glucosiduronates (glucuronides). A very small percentage is conjugated with sulfuric or phosphoric acid. Conjugated corticosteroids are water-soluble and are rapidly voided in the urine by glomerular filtration and probably also by tubular excretion. Approximately two thirds of the total plasma corticosteroids are conjugated. The formation of steroid glucuronides is not restricted to the liver, but can also take place in the kidneys. However, reduction to tetrahydro derivatives occurs almost exclusively in the liver, and generally these metabolites are also conjugated by the liver as soon as they are formed.

Only traces of unconjugated cortisol metabolites are found in the plasma. Nevertheless, steroid hormones can be metabolized extrahepatically, but only at a reduced rate. Steroid catabolism has been shown to occur *in vitro* in fibroblast cultures and also in eviscerated animals.

About 30 to 40% of the cortisol is excreted in the urine as tetrahydrocortisol and tetrahydrocortisone-glucuronide. A further 20 to 30% appears as cortol and cortolone glucuronide. The chemical nature of all the cortisol metabolites has not yet been elucidated. After the intravenous injection of a tracer dose of ^{14}C-labeled cortisol or corticosterone, 70 to 80% of the radioactivity is recovered in the urine within 24 hours and 95% within 72 hours. No radioactive CO_2 is found in the expired air. These findings indicate that the steroid skeleton itself remains intact.

The catabolic pathway of corticosterone is similar to that of cortisol, with the exception

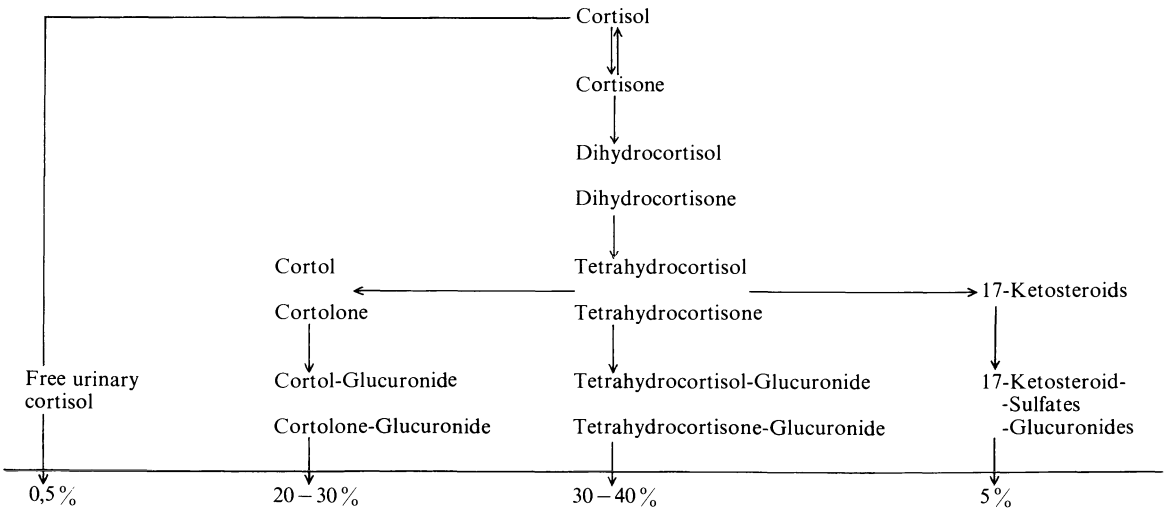


Fig. 5. Catabolism and excretion of cortisol and its metabolites

that no 17-ketosteroids are formed. The adrenals of the newborn secrete corticosterone mainly as the sulfate, and metabolites of this substance have been found in the urine.

Aldosterone is not only converted to tetrahydroaldosterone-3-glucuronide, but also to a so-called "3-oxo-conjugate" which is most probably aldosterone-18-glucuronide*. The conjugation of unaltered aldosterone is also possible outside the liver, mainly in the kidneys. Only very small amounts of free aldosterone are excreted in the urine (less than 0.5% of the aldosterone secreted), whereas the major part is excreted as tetrahydroaldosterone glucuronide (30 to 50%) and aldosterone-18-glucuronide (5 to 15%). In liver diseases and during pregnancy, the ratio of these two metabolites is altered and relatively more aldosterone-18-glucuronide is excreted.

A small percentage of ^{14}C-labeled cortisol or corticosterone given by i.v. injection is excreted in the bile. Most of it is probably reabsorbed by the intestines.

Kinetics of Cortisol Metabolism. When a pharmacological dose of cortisol or a tracer dose of radioactive – labeled cortisol is injected i.v. the logarithm of the plasma cortisol concentration or the logarithm of the specific radioactivity of the plasma cortisol decreases linearly per unit time after a short mixing period. Thus, the removal of cortisol from plasma apparently follows first-order kinetics. However, studies with large pharmacological doses of cortisol and with tracer doses of labeled cortisol lead to different results. Whereas a tracer dose is distributed only in one pool, two pools of distribution have to be assumed to explain the kinetics of a pharmacological dose of cortisol; metabolism apparently occurs in only one of them. The major part of a pharmacological dose of cortisol is probably not attached to transcortin, so that it can leave the intravascular space and only gradually returns to the blood stream.

The kinetics of aldosterone disappearance also indicate the existence of two pools of distribution.

In healthy humans, the biological half-life of plasma cortisol is on average 110 min, that of corticosterone 90 minutes, and that of aldosterone 15 and 35 min**.

The biological half-life of the adrenal cortical hormones is prolonged in liver diseases, in cachexia, in shock, myxedema, as well as during estrogen therapy and during pregnancy. It is also increased in old age, in acute polyarthritis, and in uremia. The biological half-life is decreased in thyrotoxicosis and occasionally in obesity.

The rate of removal of cortisol from the plasma can be mathematically approximated. The calculated removal-rate coefficient corresponds to a clearance and is dependent on the activity of the steroid-5-reductases in the liver, the availability of NADPH, the concentration of plasma cortisol, the binding to transcortin, and the hepatic circulation. However, the last factor appears to be negligible, since the enzymes catabolize cortisol more efficiently when the hepatic circulation is reduced.

The biological half-life of prednisone and prednisolone is almost twice as long as that of cortisol. Halogenation at position 9 delays catabolism. This at least partly explains the increased potency of synthetic fluorated or dehydrogenated steroids. The miscible metabolic pool of cortisol is 1.5 mg, that of corticosterone 0.2 mg, and that of aldosterone 0.01 mg.

Plasma cortisol varies during the day between 6 and 25 µg per 100 ml and plasma corticosterone concentration between 0.4 and 2 µg per 100 ml, whereas the aldosterone level is considerably lower and varies between 2 and 15 ng per 100 ml during unrestricted sodium intake.

7. Structure of Corticotropin (ACTH) and the Melanotropins (MSH)

Corticotropin or ACTH, which regulates the activity and growth of the adrenal cortex, is a polypeptide consisting of 39 amino acids and has a molecular weight of 4540. The structures of human, porcine, ovine, and bovine ACTH are known (Fig. 6). Ovine and bovine ACTH differ in their sequence of the amino acids from number 25 to 33. Porcine ACTH differs from bovine ACTH by having a leucine in the 31-position in place of a serin. Human corticotropin contains the same amino acids as bovine and ovine ACTH, but in a different sequence.

In 1966, SCHWYZER and SIEBER succeeded in synthesizing a complete molecule of β^{1-39} corticotropin. It has the same amino-acid sequence and the same biological activity as the natural purified porcine ACTH (110 U.S.P. units per mg). A synthetic tetracosapeptide, β^{1-24} corticotropin is commercially produced and can be used for diagnostic and therapeutic purposes. This compound contains the first

* This substance has also been refered to as the "pH 1 metabolite" in the literature, because it can be hydrolyzed by acidification to pH 1.

** Depending on the smaller or larger pool of distribution, the real nature of which is still unknown.

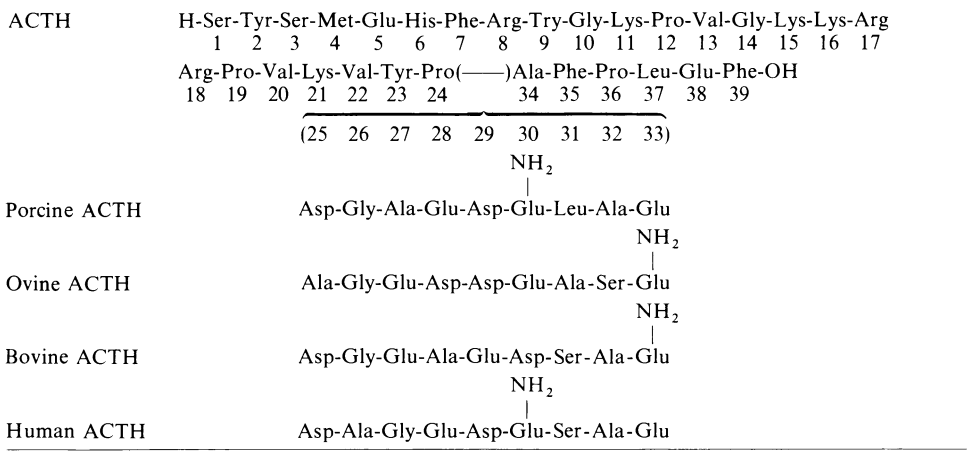


Fig. 6. ACTH structure in 4 different species. (From G. SAYERS, in: Hormones in the blood, 2nd ed., vol. 1, p. 169, ed. by C. H. GRAY and A. L. BACHARACH. London: Academic Press 1967)

24 amino acids of the N-terminal end of the complete corticotropin. Like other polypeptides containing the first 20 or more amino acids of the N-terminal end in natural sequence, it has a biological activity of 100 to 110 units per mg. Its activity per molecule is therefore somewhat lower than that of β^{1-39} corticotropin. Shorter polypeptides have a much lower ACTH activity. Peptides with less than 16 amino acids have no effect on the adrenal cortex, but have an increased MSH activity. Thus, the amino acids at the C-terminal end of the corticotropin molecule appear to inhibit MSH activity.

Recently an immunoreactive ACTH component in plasma and pituitary considerable larger than β^{1-39} ACTH, "Big ACTH" has been identified by gel filtration and ultracentrifugation. It seems to contain within it ACTH covalently linked to the carboxyl group of a basic amino acid of a larger, more acidic, peptide (YALOW, 1973).

The structure of α-MSH is the same in all mammalian species so far investigated. It consists of 13 amino acids, which are identical to the first 13 amino acids of ACTH. On the other hand, β-MSH differs from species to species. Whereas the human hormone consists of 22 amino acids, most animal hormones contain only 18 amino acids. However, the sequence of some of the amino acids also corresponds to that of corticotropin. Synthetic melanotropins have no corticotropic activity. On the other hand, some MSH activity is associated with all the corticotropins (Fig. 6).

Most of the traditional commercial ACTH preparations contained animal corticotropin extracted from pituitary tissue by glacial acetic acid and partially purified by adsorption onto oxycellulose. These preparations have now been replaced by synthetic corticotropins. Long-acting ACTH preparations contain the corticotropin as a zinc salt or dissolved in gelatine and have a prolonged effect lasting 12 to 48 hours following intramuscular injection.

Little is known about the biological inactivation and excretion of ACTH. The form in which ACTH circulates in the plasma is also unknown. ACTH has never been definitely isolated from the urine. Values reported for the biological half-life of corticotropin vary between a few minutes and several hours. The plasma concentration of ACTH in the normal human, measured with the most reliable biological assays, is approximately 0.25 mU per 100 ml in the morning and about 0.11 mU per 100 ml in the evening (NEY, 1963).

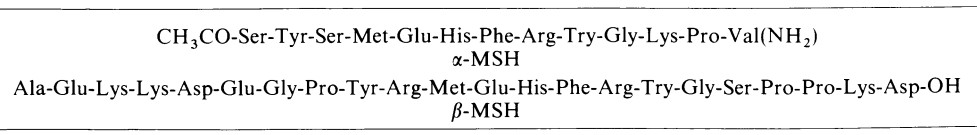


Fig. 7. Structure of human melanocyte-stimulating hormones. (From J. M. WALKER in: Hormones in the blood, 2nd ed., vol. 1, p. 161, ed. by C. H. GRAY and A. L. BACHARACH. London: Academic Press 1967)

D. Physiology

A. LABHART and J. MÜLLER

1. Biological Effects of Corticotropin

a) Action on the Adrenal Cortex

ACTH is the physiologically most important regulator of adrenal cortical function. Only in the presence of ACTH can the adrenal cortex synthesize normal amounts of glucocorticoids and androgens and secrete them into the blood stream. In the intact organism, any increase in the secretion of these hormones is due to an elevated ACTH blood level, and any reduction is due to a decreased ACTH concentration. In addition, ACTH plays a supporting role in the regulation of the secretion of the mineralocorticoid aldosterone, although the production of this hormone is controlled mainly by other factors (see p. 297). ACTH regulates the secretion of adrenal cortical hormones by stimulating their biosynthesis. In contrast to the thyroid gland and to the adrenal medulla, the adrenal cortex is unable to store its secretory products and release them on demand. The almost instantaneous elevation of the corticosteroid concentration in the adrenal venous blood induced by an intravenous injection of ACTH is also due to increased production, since the corticosteroid content of the adrenal cortex increases at the same time. Production and secretion of adrenal cortical hormones are practically identical (see Chap. I, p. 3). In addition to regulating the production of steroid hormones, ACTH also controls the size and growth of the adrenal cortex: excessive secretion of ACTH leads to hyperplasia, while failure leads to atrophy. The fact that synthetic ACTH also stimulates adrenal growth and corticosteroid production makes the existence of a separate adrenal growth-promoting hormone appear unlikely.

Experiments with radioactive-labeled precursors of corticosteroids indicate that ACTH stimulates the synthesis of steroid hormones mainly at the stage of the conversion of cholesterol to pregnenolone (STONE, 1954; KARABOYAS, 1965). This step is common to the biosynthesis of all steroid hormones. It is still uncertain whether the effect of ACTH is mediated by the activation of one of the three enzymes involved in this conversion or by an increased availability of the essential coenzyme NADPH. However, intracellular transport mechanisms or morphological changes of the mitochondria may also play an important part. Whereas an intravenous injection of ACTH leads to immediate cholesterol depletion of the adrenal cortex, long-term ACTH administration induces an increased cholesterol content, indicating that ACTH may also stimulate cholesterol biosynthesis. Prolonged ACTH administration also leads to enhanced 11β-hydroxylase activity (GRANT, 1957).

The exact biochemical mechanism of action of ACTH is controversial. According to the classic theory of HAYNES and BERTHET (1957), ACTH stimulates the formation of cyclic AMP. Cyclic AMP activates phosphorylase, which in turn mediates the conversion of glycogen to glucose-1-phosphate. Glucose-1-phosphate is converted to glucose-6-phosphate by phosphoglucomutase. Metabolism of glucose-6-phosphate by the hexose monophosphate shunt results in the production of NADPH, a coenzyme necessary to several steps of steroidogenesis. More recent evidence does not confirm this theory *in toto* (HILF, 1966). However, it has been amply demonstrated that ACTH acutely raises the cyclic AMP content of adrenal cortical tissue. Moreover, cyclic AMP simulates the steroidogenic effect of ACTH on perfused adrenal glands or incubated adrenal tissue.

ACTH also promotes protein synthesis in the adrenal cortex. This effect, however, seems to be related to the chronic growth-stimulating effect rather than to the acute stimulation of steroidogenesis, but ACTH induces a rapid increase of the ribonucleic acid concentration in the adrenal tissue (FIALA, 1956). The corticosteroid-stimulating effect of ACTH is blocked *in vivo* and *in vitro* by puromycin and chloramphenicol (FERGUSON, 1963; GARREN, 1965), which indicates that the acute effects of ACTH are also dependent on the integrity of protein synthesis.

The significance of the high content of ascorbic acid in the adrenal cortex and its rapid disappearance under the influence of ACTH is unknown. Biosynthesis of steroid hormones and the adrenal responsiveness to ACTH are normal in patients with scurvy.

b) Extra-Adrenal Effects

In addition to its action on the adrenal cortex, ACTH exerts specific effects on other tissues, which are demonstrable particularly *in vitro*, but sometimes also in adrenalectomized animals *in vivo*. One of these extra-adrenal actions is the stimulation of the melanocytes, which has already been mentioned (see p. 295).

The best-known extra-adrenal activity of ACTH is its lipolytic action on adipose tissue. In extremely low concentrations it stimulates the hydrolysis of the triglycerides. This effect is probably due to activation of a specific lipase

of adipose tissue, which is not identical to lipoprotein lipase. This action of ACTH is also mediated by cyclic AMP. ACTH as well as catecholamines or glucagon stimulate lipolysis acutely, whereas growth hormone has a delayed action; its lipolytic activity is probably due to the stimulation of lipase synthesis (FAIN, 1965, 1967; VAUGHAN, 1965).

2. The Regulation of Adreno-Cortical Hormone Secretion

a) The Regulation of Aldosterone Secretion

The complex physiological and biochemical control mechanisms which regulate the secretion of aldosterone are only partially known. The original assumption that a specific aldosterone-stimulating hormone regulated the secretion of aldosterone in a similar manner to the way ACTH regulates the secretion of cortisol led to the discovery of the multiple functional relations between the renin-angiotensin system and aldosterone production by the adrenal cortex (GROSS, 1958; DAVIS, 1961; GENEST, 1961; LARAGH, 1960). Angiotensin II does indeed have many properties of a trophic aldosterone-stimulating hormone. Very low doses of angiotensin II stimulate the aldosterone production by isolated adrenal glands *in vivo* and *in vitro* without affecting the production of glucocorticoids. In several diseases associated with secondary aldosteronism the increased aldosterone secretion is very probably due to increases in plasma renin and angiotensin II concentrations. Plasma renin activity is elevated in Addison's disease and depressed in primary aldosteronism. Nevertheless, the analogy between angiotensin II and ACTH is incomplete and the regulation of aldosterone secretion differs from the regulation of cortisol secretion in several respects.

1. Whereas ACTH is the only substance which can stimulate the secretion of glucocorticoids in physiological concentrations, a number of different humoral factors can stimulate aldosterone production.

2. Aldosterone production can be regulated at different stages of the biosynthetic pathway.

3. The regulation of plasma aldosterone concentration does not include a direct negative feedback mechanism.

4. There may be wide species differences in the regulation of aldosterone secretion.

The following factors have been found to stimulate the biosynthesis of aldosterone by incubated adrenal tissue or in perfused adrenal glands directly.

angiotensin II,
elevated potassium concentration,
reduced sodium concentration,
ACTH,
serotonin.

During short-term experiments, all these factors stimulate aldosterone biosynthesis by enhancing the conversion of cholesterol to pregnenolone in the cells of the zona glomerulosa (MÜLLER, 1966). Most of these stimulators act exclusively or preferentially on zona glomerulosa cells, whereas ACTH also acts on the inner zones, thus stimulating the production of glucocorticoids and androgens (MÜLLER, 1970).

In man and in animals, the aldosterone-stimulating effect of ACTH is limited to a few days, whereas the cortisol production is stimulated for however long ACTH is given. The mechanism of this escape of aldosterone secretion from the stimulating effect of ACTH is as yet unknown. Hypophysectomy is followed by an initial decrease of 50–90% in the aldosterone secretion rate, but a return to normal values can be observed after a short period.

A decreased sodium concentration in adrenal arterial plasma directly stimulates aldosterone output. On the other hand, experimental hyponatremia induced by pitressin injection and increased water intake does not enhance aldosterone secretion, probably because renin secretion is simultaneously suppressed by the hypervolemia. By contrast, an increased potassium intake stimulates aldosterone production although plasma renin activity decreases under these experimental conditions (VEYRAT, 1967).

Serotonin directly stimulates aldosterone biosynthesis, but this effect has as yet been demonstrated only *in vitro* (ROSENKRANTZ, 1959; MÜLLER and ZIEGLER, 1968) and it is not known whether this substance is of any physiological significance in the regulation of aldosterone secretion.

Table 2 presents experimental or pathophysiological situations regularly associated with an increased or reduced aldosterone secretion rate. A reduction in the circulating blood

Table 2. Control of aldosterone secretion

Aldosterone secretion	
Stimulating	Suppressing
Sodium depletion	Sodium loading
Potassium loading	Potassium depletion
Hypovolemia (hemorrhage)	Hypervolemia (plasma infusion)
Diminution of cardiac output	Increase of cardiac output
Diminution of renal blood flow	Nephrectomy
Upright posture	Supine posture
Constriction of the inferior vena cava	

volume is probably the stimulus leading to an increased aldosterone secretion, not only after hemorrhage but also in diseases characterized by edema (cirrhosis of the liver, nephrotic syndrome). Hypovolemia leads to an increase in renin production and an elevated level of plasma angiotensin II which then stimulates the aldosterone secretion. The receptors presumably lie in the kidneys, but their exact locations is unknown. They may be located in the juxtaglomerular cells or in the macula densa cells of the distal tubules, which are in the immediate vicinity of the juxta-glomerular cells. It is not known whether these are primarily pressure-, volume-, flow-, or chemo-receptors. An increase in aldosterone secretion in response to upright posture is also secondary to elevated renin production, which is probably mediated by the sympathetic nervous system.

A number of different factors appear to be involved in the adaptation of aldosterone secretion to alterations in sodium intake or sodium balance. A significant decrease in the serum sodium concentration is only observed in severe sodium deficiency. Even a moderate sodium deficiency, however, leads to hypovolemia and thus to an increase in renin production. However, in sodium-deficient sheep an increased aldosterone secretion rate is maintained for many hours after bilateral nephrectomy (BLAIR-WEST, 1964). In addition, the aldosterone-stimulating effect of angiotensin II, potassium ions, and ACTH is considerably more potent in sodium deficiency than in sodium balance, which is due partly to an increased sensitivity of the adrenal cortex and partly to increased activity of the enzymes involved in the conversion of corticosterone to aldosterone (MARUSIC, 1967; MÜLLER, 1968; DAVIS, 1968). Certain parts of the central nervous system also seem to be involved. In sodium-deficient sheep, midcollicular decerebration impairs the rapid decrease of aldosterone secretion in response to resumed sodium intake.

b) Regulation of the Plasma Cortisol Concentration

In the human, the cortisol secretion is directly dependent on the plasma concentration of corticotropin. The cortisol concentration in the plasma and the excretion of urinary 17-hydroxycorticosteroids are linearly correlated to the logarithm of the ACTH concentration in the plasma. The adrenal cortex is maximally stimulated by an ACTH concentration of 3 mU/100 ml. There is a circadian rhythm of ACTH concentration with an average value of 0.25 mU/100 ml at 6 a.m. and a fall to 0.1 mU/100 ml at 6 p.m. (Fig. 8).

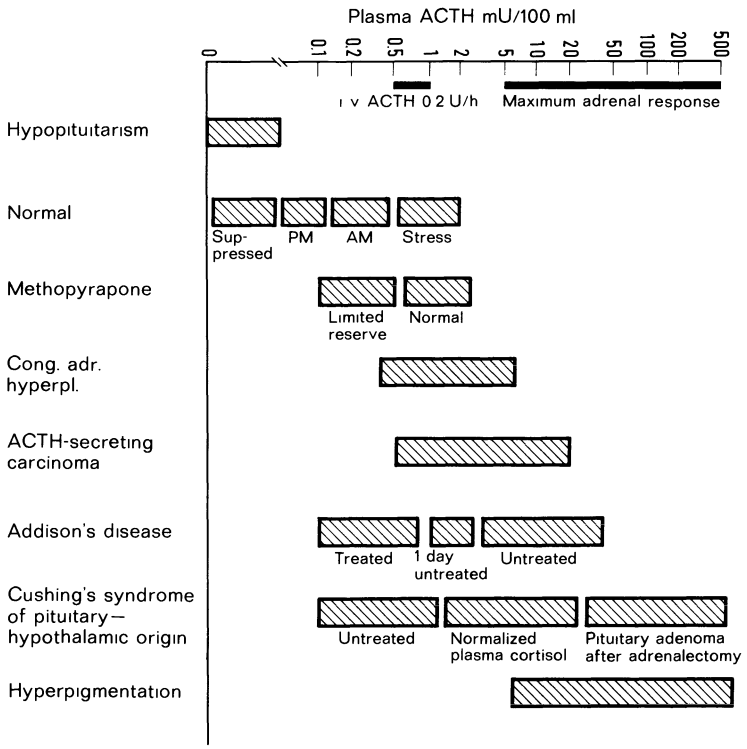


Fig. 8. Plasma ACTH in different clinical conditions. (From LIDDLE, ISLAND, and MEADOR, 1962)

An intravenous infusion of 0.25 units over 8 hours causes a significant rise in urinary corticosteroid excretion measured every 4 hours. A maximum response is elicited by 1 unit. Higher doses have a more prolonged effect. A basal ACTH secretion rate of 0.5 to 1 units per day has been calculated from infusion experiments. The rise in plasma cortisol in the morning is elicited by a dose of ACTH corresponding to a secretion rate of 4 units per day. The approved standard dose for diagnostic and therapeutic purposes is 25 units given in an intravenous infusion over 8 hours.

The plasma concentrations of cortisol or corticosterone are regulated by a control system which can be described in cybernetic terms as a closed-loop control with a negative feedback and a variable set-point. Most of the experimental and clinical data available on cortisol and corticosterone concentrations, secretion rates, metabolism, and excretion easily fit into a theoretical model of this type (YATES and URQUHART, 1962). Peripheral stimuli promoting the secretion of cortisol are integrated in the hypothalamus, elicit the release of a neurohormone (CRF, corticotropin releasing factor), which in turn mediates the secretion of corticotropin by the anterior pituitary gland. ACTH then stimulates the production and secretion of cortisol by the adrenal cortex. When the plasma cortisol concentration has reached the desired level the hypothalamic secretion of CRF is turned off, to keep the plasma cortisol concentration constant. As soon as the concentration of cortisol in the plasma decreases due to degradation by the liver, the inhibitory effect on the hypothalamic regulator ceases, CRF is again secreted, ACTH is released, and more cortisol is produced to restore the plasma concentration (Fig. 9).

This closed-loop control functions very rapidly. Cortisol can be secreted within seconds in response to a stimulus. The negative feedback control is effective within 15 sec.

Even the increased plasma cortisol concentration following acute stress and adrenal hyperplasia following chronic stress are consistent with the hypothesis of a closed-loop control system, if a variable set-point of the hypothalamic regulator is assumed. In chronic stress the system works in the normal way, maintaining an increased plasma cortisol level and an elevated secretion rate. However, an increased ACTH secretion in response to the stress of a surgical operation cannot be inhibited even by high doses of dexamethasone (LIDDLE, 1960). In a more recent publication, YATES (1967) therefore extended his model to include the possibility of a CRF secretion independent of negative feedback control. In this new model, the afferent stimuli are divided into a "corticosteroid-sensitive" and a "corticosteroid-insensitive" class.

Most experimental evidence indicates that the cortisol-sensitive regulator is located in the hypothalamus. Cortisol implants into closely defined sites of the median eminence and the post-optical region inhibit the stress reaction in animal experiments. Although certain animal experiments have suggested that cortisol has a direct inhibitory effect on the pituitary (DE WIED, 1961), cortisol implants are less effective in the pituitary gland than in the hypothalamus. Experiments using a microelectrophoretic technique and involving recordings of the action currents of individual neurons have recently suggested that the inhibitory action of the glucocorticoids is registered by the neurons in the upper central gray matter around the 3rd ventricle (RUF, 1967). A direct

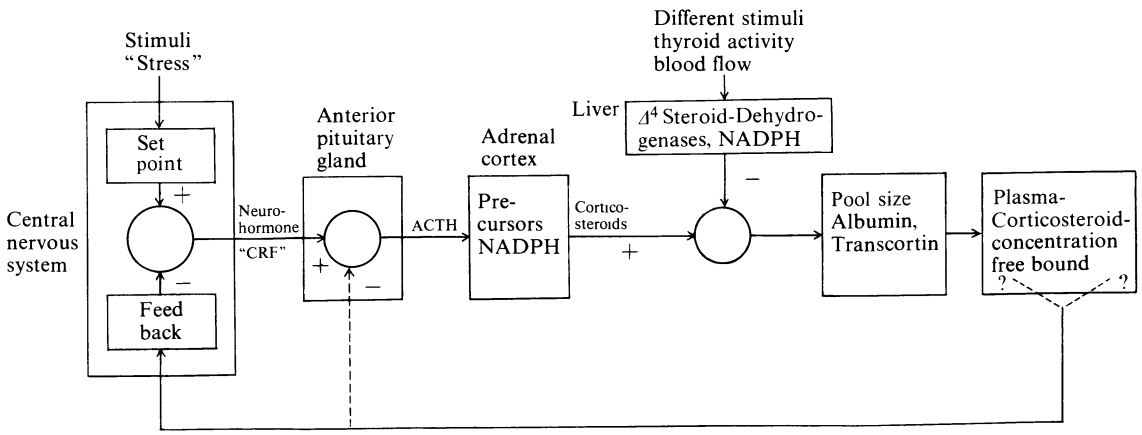


Fig. 9. Closed-loop regulation of the hypothalamic-pituitary adrenocortical system with variable set-point. (Simplified from YATES and URQUART, 1962)

inhibitory effect of cortisol on the adrenal cortex is only manifest at extremely high levels and is of no physiological importance.

c) The Hypothalamic ACTH-Releasing Neurohormone "CRF" (Corticotropin-Releasing Factor)

Information is transmitted from the hypothalamus to the pituitary gland by a humoral signal, since the functional unity is dependent on an intact portal venous system in the pituitary stalk, whereas there are no nervous connections to the anterior pituitary gland. Presumably, the neurohormones are formed in the tuberoinfundibular nucleus and particularly in the median eminence. The exact structure of the natural neurohormone CRF is still unknown. Several attempts to isolate the hormone have failed, mainly because the *in-vivo* and *in-vitro* bioassays used have not been sufficiently specific. Fractionation of hypothalamic and posterior pituitary extracts has yielded two types of active polypeptides which stimulate the production of ACTH *in vivo* and *in vitro*:

α-CRF is less active. It is a polypeptide consisting of 10 amino acids, and is structurally related to α-melanotropin. It is not known whether this substance is hormonally active or promotes ACTH synthesis by being one of its structural elements. A synthetic heptapeptide proved to have CRF activity *in vitro*, but not *in vivo*.

β-CRF, on the other hand, which is obtained from crude posterior pituitary extracts, is active even at extremely low concentrations and has a structure similar though not identical to that of vasopressin. Vasopressin itself exerts a CRF-like action, but only in high concentrations. In the intact animal, it acts predominantly as a stimulator of endogenous CRF secretion.

d) Suppressive Action of the Glucocorticoids on the Hypothalamus

The inhibitory effect of different natural and synthetic adrenal cortical hormones on the hypothalamus generally parallels their glucocorticoid and anti-inflammatory activity. Thus, among the synthetic corticosteroids, prednisone and prednisolone are 5 times more active and dexamethasone 40 times more active than cortisol in suppressing ACTH secretion.

Certain synthetic 21-deoxysteroids have a strong suppressive effect on ACTH secretion, whereas their action on the eosinophils and on carbohydrate metabolism is very weak. Thus, a dissociation of the various activities of synthetic steroids is possible at least in theory.

The administration of high doses of cortisol and cortisone leads to gradual functional impairment and atrophy of the adrenal cortex with a regressive transformation similar to that observed after hypophysectomy (see p. 95). Adrenal atrophy is prevented by the simultaneous administration of corticotropin. In man, a daily dose of 75 mg of cortisone acetate completely suppresses ACTH secretion. Adrenal atrophy develops rapidly. The adrenal response to ACTH is reduced even after a few days and is completely absent after a long-term therapy with high doses of cortisone. After withdrawal of cortisone therapy, the adrenals can only maintain a basal cortisol secretion, and are unable to increase their cortisol output in response to stress. Death due to adrenal failure secondary to cortisone therapy has been described. Adrenal atrophy due to either hypophysectomy or cortisone therapy is generally reversible. Intensive therapy with ACTH for two to four days usually restores adrenal cortical function to normal, even after many years of suppression (see secondary insufficiency, p. 325 ff.).

Certain C_{19}- and C_{21}-steroids have the property of preventing adrenocortical atrophy in rats treated with cortisone. The mechanism of this effect is unknown. It brings to mind the maintenance of the tubular testicular apparatus under testosterone therapy after hypophysectomy. Most of the active compounds are androgens. However, the adrenal atrophy preventing activity is not parallel to their androgenic activity. The effect was not demonstrable in man. It seems to be different from the general anabolic activity common to all androgens.

e) Circadian Rhythm of Plasma Cortisol

In the absence of extraordinary stress, the plasma cortisol concentration of healthy humans varies within certain limits in a fixed diurnal rhythm (5 to 25 μg per 100 ml). Plasma cortisol reaches its peak in the early hours of the morning, between 6 and 9 a.m., and falls continuously

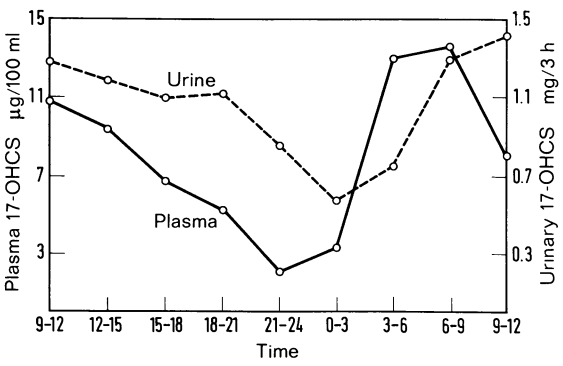


Fig. 10. Diurnal variation of plasma and urinary 17-hydroxycorticosteroids (17-OHCS). Mean values of 8 normal subjects. (From C. L. Cope, 1965; according to R. P. Doe, E. B. Flink, M. G. Goodsell, 1956)

during the day, reaching its lowest level around midnight (Fig. 10). At about 2 a.m. it rises again rapidly. When the urinary excretion of 17-hydroxycorticosteroids is measured every hour, the highest values are observed before noon, the lowest after midnight. The blood eosinophil count follows this rhythm in the opposite direction with a four-hour delay. It is highest during the night, and lowest in the morning. Plasma corticosterone levels follow the same circadian rhythm as plasma cortisol concentrations.

This rhythm is independent of light perception (occurring in blind subjects and in miners) and of physical activity, but is related to the sleep rhythm. After weeks of continuous night work and sleep during the day the rhythm can adapt to the new conditions (ORTH, 1967).

The circadian rhythm is controlled by the central nervous system and is altered or absent in brain diseases. It is mediated by ACTH secretion and disappears following prolonged stimulation or long-term administration of cortisone. The adrenals are more responsive to a standard dose of ACTH during the day than after midnight. This difference, however, disappears when a small amount of ACTH is given before midnight.

In Cushing's syndrome, the diurnal variation is characteristically absent. Abnormalities or absence of the circadian rhythms are also observed in certain psychoses, in cardiac diseases, and in renovascular hypertension. Elevated levels of plasma cortisol due to an increase of transcortin during estrogen therapy or during pregnancy, on the other hand, are characterized by a pronounced diurnal variation.

3. Biological Actions of the Adrenal Cortical Hormones

The physiologically most important adrenal cortical hormones can be divided into three groups according to their biological activity.

Hormones acting predominantly on sodium and potassium balance are called *mineralocorticoids* after SELYE, and hormones affecting carbohydrate and protein metabolism *glucocorticoids*. In both sexes, the adrenal cortex produces hormones with the properties of the male gonadal hormones: the *adrenal androgens*. It is not known whether their weak anabolic, i.e. protein synthesis-promoting, effect is of any physiological significance, or whether they play some part in maintaining normal libido in the woman (see p. 310).

The action of the individual hormones is not always restricted to one type of functions, and their activities can overlap. Cortisol, the most important glucocorticoid in man, also has a weak mineralocorticoid activity; some of its metabolites are slightly androgenic and thus also anabolic. The effect of glucocorticoids on carbohydrate metabolism is generally, but not always, paralleled by their anti-inflammatory, eosinopenic, thymolytic, and ACTH-suppressive activities.

Biological activity is always related to chemical structure. All steroids with mineralocorticoid or glucocorticoid activity are pregnane derivatives with 21 C-atoms, a 3-keto group, a double bond between C-4 and C-5 and an α-ketol side chain at C-17. The glucocorticoid activity is dependent on a hydroxyl or keto group at C-11, and is enhanced by a 17α-hydroxyl group. Adrenal androgens are derived from androstane; they have 19 C-atoms and a keto- or hydroxyl group at position 17. The structural basis of mineralocorticoid activity is still poorly understood.

In theory all the cells in the organism are under the influence of all the adrenal cortical hormones. However, whereas the tubular renal apparatus is the main target organ of the mineralocorticoids, the metabolism of all the cells is considerably influenced by the glucocorticoids.

The effects and activities of natural adrenal cortical hormones and synthetic derivatives of therapeutic value are listed in Tables 3 and 4.

a) Mineralocorticoids

α) Aldosterone

By regulating sodium and potassium balance, aldosterone controls the volumes and the cationic composition of the extracellular and intravascular fluids. Aldosterone receptors are found in the epithelia which are actively transporting sodium and potassium ions, and the most important target organs of aldosterone are the kidneys, the gut, the salivary and the sweat glands. In the human, its main action is on the tubular apparatus of the kidneys. Aldosterone promotes reabsorption of sodium in the distal tubule and leads to increased excretion of potassium and hydrogen ions. Although sodium reabsorption does not quantitatively correspond to potassium and hydrogen excretion, and the ratio of potassium to hydrogen ions is variable, the effect of aldosterone virtually consists of an exchange of sodium ions against potassium and hydrogen ions. According to the investigations of KASSIRER (1967), aldosterone does not directly promote the excretion of hydrogen ions. An additional effect of aldosterone on proximal tubular sodium reabsorption, sug-

gested by indirect evidence (WIEDERHOLT, 1965; HIERHOLZER, 1966), is still controversial. Aldosterone also promotes the excretion of magnesium and ammonium ions. The increased formation of ammonium in aldosteronism is, however, a result of the hypokalemia.

In primary aldosteronism or after prolonged administration of high doses of aldosterone (in man, 1 to 3 mg/day for 2 weeks), a compensatory mechanism for sodium retention, but not for potassium loss, can be observed. This so-called escape phenomenon only concerns the kidneys and does not involve the salivary and sweat glands. It is absent in diseases associated with edema (nephrosis, cirrhosis of the liver). The escape phenomenon is partly due to hemodynamically increased glomerular filtration. However, since it is also effective when glomerular filtration is decreased, other mechanisms must be involved. The escape phenomenon may be due to the action of an unidentified sodium excretion-promoting hormone, the so-called third factor, originating in the hypothalamus or the kidneys (BRICKER, 1967; LICHARDUS, 1966; JOHNSTON, 1967; MARTINEZ-MALDONALDO, 1967; DÖLLE, 1968; FREAZIER, 1968).

The mucosal epithelium of the toad bladder is functionally similar to the mammalian distal tubular epithelium. It reabsorbs sodium and water from the urine and thus initiates their transport into the interstitial fluid. This activity can be reproduced in vitro and can be stimulated by vasopressin or aldosterone (LEAF, 1955; CRABBÉ, 1961). However, there is no exchange between sodium and potassium in this tissue. When aldosterone is added to the incubation medium in extremely small amounts (3×10^{-10} to 10^{-7} mol per liter), there is a dose-dependent increase in sodium transport after a latency period of 1 to 2 hours. The action of aldosterone is associated with binding of the hormone to the nuclei of the mucosal epithelium cells, which can be demonstrated by autoradiography (PORTER, 1964). Binding occurs within 30 to 45 min. Aldosterone can be competitively dissociated from the active binding sites by other steroids with mineralocorticoid activity, as well as by aldosterone antagonists (progesterone, spironolactone). The inhibitory effect of certain antibiotics suggests that the action of aldosterone on sodium transport involves the synthesis of a deoxyribonucleic acid-dependent ribonucleic acid. It is assumed that under the influence of this RNA a specific short-lived protein is synthesized, which facilitates the entry of sodium into the mucosal cells. Stimulation of the sodium transport by aldosterone requires the presence of specific energy-supplying substrates (pyruvate, acetoacetate). Aldosterone is chemically unaltered while exerting its biological effect. Although the mammalian kidney is only partly comparable to the toad bladder, experiments with this model have yielded valuable information on a general mode of action of the mineralocorticoids.

Outside the kidneys, aldosterone also inhibits sodium excretion and promotes potassium excretion in the salivary and the sweat glands and in the gut. However, it is uncertain whether aldosterone can also directly influence the sodium and potassium transport across the plasma membranes of other cells. It is also uncertain whether aldosterone has a direct vasotonic effect and whether its digitalis-like positive inotropic action on the myocardium is physiologically important.

In physiological doses, aldosterone has no effect on carbohydrate metabolism, no anti-inflammatory properties, no suppressive effect on ACTH secretion, and no clear-cut eosinopenic effect, and it does not alleviate pigmentation in Addisonian patients. Due to the low aldosterone concentration in the plasma, its glucocorticoid activity, which is only approximately one third that of cortisol, is not apparent.

According to the normal daily secretion rate, the normal maintenance dose for an adrenalectomized man is 100 to 300 μg given subcutaneously or intramuscularly, 1 to 2 mg taken sublingually or 3 mg taken orally. Aldosterone is, however, seldom used for chronic maintenance therapy, since an equivalent oral dose of 0.1 mg of 9α-fluorocortisol is much more convenient and economical.

Aldosterone deficiency leads to hyperkalemia, hyponatremia, hypovolemia, hypotension, and metabolic acidosis, and aldosterone excess to hypokalemia, metabolic alkalosis, hypokalemic dysfunction of the kidney tubules with hyposthenuria, and hypertension. Aldosterone has no direct negative feedback effect on aldosterone production by the adrenal cortex or on renin production by the kidneys. However, an indirect feedback axis leads from sodium retention to hypervolemia and to the suppression of renin production. Long-term administration of mineralocorticoids results in atrophy of the zona glomerulosa of the adrenal cortex.

β) Deoxycorticosterone (Cortexone)

Deoxycorticosterone is a biological precursor of corticosterone and aldosterone and is only found in traces in the adrenal venous blood. It generally has the same mineralocorticoid effects as aldosterone, but is 20 to 40 times less active. On the other hand, it has no glucocorticoid activity.

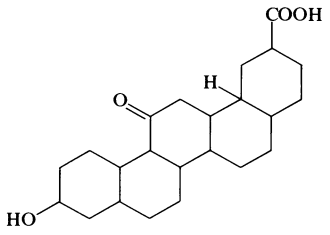


Fig. 11. Structure of β-glycyrrhetinic acid

Glycyrrhicic acid from licorice juice has a pronounced mineralocorticoid activity, but no glucocorticoid activity. Excessive intake, e.g. in the form of cough drops or in certain soft drinks, can lead to a disease very similar to primary aldosteronism (Fig. 11).

γ) Mineralocorticoid Antagonists

A natural adrenal cortical hormone which promotes sodium diuresis in physiological concentrations by inhibition of aldosterone action on the renal tubules has so far not been discovered in man. Progesterone in high doses corresponding to the secretion rate in advanced pregnancy, 17α-hydroxyprogesterone and testosterone can competitively inhibit the action of aldosterone on the kidneys and other target organs. By contrast, 3β,16α-dihydroxyallopregnane-20-one is inactive in man. Synthetic steroids of the spironolactone group are more effective aldosterone antagonists. They act only in the presence of mineralocorticoids and have no natriuretic effect in adrenalectomized animals not treated with mineralocorticoids (Fig. 12).

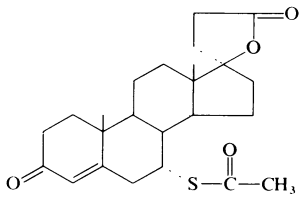


Fig. 12. Structure of the aldosterone antagonist spironolactone (aldactone)

Triamterene, a pteridine derivative, has a similar action to spironolactone, but is also effective in the absence of mineralocorticoids. It is, therefore, not a true aldosterone antagonist.

The substance 3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)-pyridine (SU 9055, Ciba) directly inhibits 18-hydroxylation and leads to a decrease in aldosterone secretion and to an increase in corticosterone secretion. Heparin and natriuretic heparinoids act by indirect inhibition of aldosterone biosynthesis. The reader is referred to p. 302 for a discussion on the natriuretic "third" factor, which may be a hormone.

b) Glucocorticoids

α) Corticosterone

Corticosterone is a natural adrenal cortical hormone with glucocorticoid and mineralocorticoid properties. When taken orally, its activity is very weak, but it is more effective when administered parenterally. It has a weak aldosterone-like effect on sodium and potassium balance. It has a slight effect on glucose tolerance and protein metabolism. Eosinopenic and anti-inflammatory activities are absent.

β) Cortisol

Although the text below only refers to cortisol, cortisone and numerous synthetic corticosteroids have the same physiological and pharmacological activities.

The metabolic action of cortisol supplies glucose to the body by the transformation of proteins. Thus, the overall effect of cortisol is an ergotropic one, directing metabolism from a phase of growth and storage towards increased physical activity and energy consumption. Very little is known about any cellular or molecular base of the mode of action. The reader is referred to the reviews by WEISSMANN (1964), WEBER (1968), and RAY (1968).

1. Effect on Carbohydrate Metabolism. Cortisol stimulates gluconeogenesis, i.e. the formation of carbohydrates from amino acids by mobilization and degradation of protein. Gluconeogenesis makes it possible for blood glucose levels to be kept constant during fasting without depletion of the glycogen stores of the liver.

Newly formed glucose is partially converted to fat under the influence of insulin. The glucose turnover rate is thus accelerated, the blood sugar elevated by 10 to 20 mg%, and glucose tolerance and insulin sensitivity decrease. However, carbohydrate metabolism is still firmly controlled as long as the pancreatic islets are functionally intact.

So-called "steroid diabetes", i.e. a benign form of diabetes with a low incidence of ketosis, a low sensitivity to insulin and a decreased renal threshold for glucose, develops in only 20% of patients treated with high doses of cortisone. It is striking that 20 to 25% of the Western population are estimated to be predisposed to diabetes mellitus.

Gluconeogenesis is maintained mainly by the liver with slight contributions by the kidneys and the intestinal epithelia.

The mechanism of action is not clear. Under the influence of cortisol, the content of certain

glycolytic enzymes, as well as of glutamate-pyruvate-transaminase, fructose-1,6-diphosphatase, glucose-6-phosphatase among others, increases in the liver. However, this may be the consequence of increased availability of substrates rather than the primary effect of cortisol. The effect on gluconeogenesis is not blocked by inhibitors of protein synthesis, such as actinomycin D (for literature see RAY, 1968).

Cortisol increases the very low physiological glycosuria (by 50 to 200 mg/24 hours) by simultaneously raising blood sugar and glomerular filtration rate. On the other hand, glucose reabsorption in the proximal tubule is not inhibited. The maximum capacity for tubular glucose reabsorption remains unaltered (FROESCH, 1958).

Cortisol apparently also has a permissive effect on the glycogenolytic action of adrenaline; i.e. normal glycogenolysis due to adrenaline is only possible in the presence of cortisol.

An earlier assumption that cortisol inhibits peripheral glucose degradation has not been confirmed. The increased level of pyruvic acid in the blood is due to increased glucose turnover rather than to decreased catabolism. Glucose uptake is inhibited only in the isolated adipose tissue (see below).

2. Effects on Protein Metabolism. Cortisol leads to a negative nitrogen balance which can be alleviated by a high intake of carbohydrates or protein. As demonstrated by studies with labeled albumin, protein degradation is increased, and is not fully compensated by a simultaneously increased protein synthesis. The synthesis of ribonucleic acids and of certain enzymes involved in protein metabolism in the liver is accelerated. Protein degradation also occurs outside the liver.

Cortisol increases amino-acid excretion. The kidney threshold for amino acids remains constant, but the plasma concentration increases. Cortisol promotes the excretion of uric acid without affecting its serum concentration. According to studies with isotopically labeled uric acid, this is due to an increase in uric acid clearance.

The negative nitrogen balance induced by the catabolic effect of glucocorticoids can lead to bone, muscle or skin damage, as well as to growth impairment in children. Degradation of bone matrix and osteoporosis, muscular waste, atrophy and vulnerability of the skin are manifestations of the negative protein balance induced by long-term therapy with high doses of cortisol or cortisone, or by Cushing's syndrome. Experiments with thymocytes have yielded valuable information about the mode of action of cortisol through RNA and DNA (see p. 305) (MAKMAN, 1967).

3. Effect on Lipid Metabolism. Cortisol has an indirect effect on lipid metabolism by its action on carbohydrate metabolism. Adrenalectomy is followed by loss of appetite and thus by lipid depletion in the organs. After exhaustion of the carbohydrate stores, fat is used mainly as a source of energy, because in the absence of glucocorticoids proteins are not accessible. *In vitro*, cortisol inhibits glucose uptake by isolated adipose tissue. This decreases the rate of glycerophosphate formation and thus the re-esterification of free fatty acids to triglycerides, resulting in an increased output of free fatty acids. In the intact organism, this effect is overcompensated by an increased insulin secretion leading to fat deposition, as for instance in Cushing's syndrome (see p. 340ff.).

4. Effects on Electrolytes. Cortisol acts in a similar manner to the mineralocorticoids on urinary and plasma electrolytes by promoting sodium retention and potassium excretion, but is approximately 1000 times less potent than aldosterone. This effect, however, is dependent on the mode of administration, the dosage, and the duration of cortisol administration. In low doses, cortisol can promote sodium diuresis. Cortisol and cortexone, given simultaneously in low doses, have an additive, and not a competitive effect on electrolytes. The serum potassium may be elevated despite an increased excretion of potassium in the urine, which indicates that high doses of cortisone primarily promote the transport of intracellular potassium into the extracellular space. In the healthy human, the effect is most marked early in cortisone therapy. With continued administration, it gradually decreases and may finally become reversed, resulting in sodium loss and potassium retention. Potassium retention may be due to increased glycogen storage in the liver. The loss of intracellular potassium is compensated by the uptake of sodium and hydrogen ions from the extracellular space, which leads to metabolic alkalosis. The excretion of H-ions is not directly influenced by cortisol, but depends on phosphate excretion. Nor is any promoting effect of cortisol on bicarbonate reabsorption demonstrable. The alkalosis is therefore only a result of altered potassium distribution.

Calcium and phosphate excretion are stimulated by cortisol. They exceed the expected values calculated from the nitrogen balance. This is partially due to increased phosphate clearance. In addition, cortisol promotes calcium excretion into the gut, and inhibits

intestinal and renal tubular reabsorption and calcium mobilization from the bones. In healthy subjects, this hypocalcemic action is effectively compensated by the normal mechanisms regulating calcium homeostasis. However, in various diseases, cortisol and its derivatives can lower an increased blood calcium level. Hypercalcemia is occasionally observed in adrenal cortical failure (see p. 318f.).

5. Effect on Water Homeostasis. The inability to achieve rapid diuresis in response to a water load has long been recognized as a symptom of Addison's disease and has been used as a diagnostic test (see p. 391). Normal rapid water diuresis is restored by cortisol therapy but not by treatment with mineralocorticoids such as cortexone or aldosterone.

The deficient water diuresis in adrenal failure is not due to delayed absorption from the gastrointestinal tract, but rather to insufficient excretion through the kidneys. The diuretic effect of cortisol is partly due to increased glomerular filtration. It also acts permissively on water permeability of the distal tubular segments during diuresis. An effect on vasopressin action is probable (see Chap. IV, p. 49).

6. Effects on Tissues. Cortisol does not act uniformly on all the tissues, since the sensitivity of the cells to cortisol varies according to their origin and their stage of development.

In general, tissues arising from entoderm are little affected, whereas mesenchymal and ectodermal epithelial components are strongly influenced. The action of cortisol depends very much on the species, and information obtained from animal experiments can only be extrapolated to human pathophysiology with reservations.

Some tissues are directly affected by cortisol, and others indirectly via metabolic changes. Direct effects have been observed in connective tissue, adipose tissue, the lymphatic system, and blood, and predominantly indirect effects in muscle and bone. On the other hand, connective tissue, in particular the fibroblasts, can inactivate cortisol by conversion and degradation (see p. 293).

Connective Tissue. Undifferentiated mesenchymal tissues are particularly sensitive, in contrast to mature tissues such as granulation tissue of scars, which is hardly affected. Growth of fibroblasts is inhibited. Collagenous fibers are converted into a homogeneous mass. Basic substance is reduced, and its content of acidic mucopolysaccharides is decreased. Elastic fibers are not affected, but appear to be more numerous due to the loss of other tissue components.

The inhibitory effect of cortisol on connective tissue is most pronounced in inflammatory reactions with proliferation of granulation tissue. However, exsudation is affected as well as proliferation. Vascularization is inhibited, capillary permeability is decreased. The infiltration of leukocytes and phagocytes is reduced. Experimental peritoneal adhesion can be prevented by cortisol. Cortisol protects the lysosomes against various noxious influences (WEISSMANN, 1964).

Whenever inflammation is purposeful as in resistance to infection, the anti-inflammatory effect of cortisol is harmful. However, it is of greatest therapeutic importance when the organism might be damaged by the inflammatory process, as in allergic conditions. The anti-inflammatory activity of cortisol is only manifest at doses well above the physiologic range of 20 to 40 mg per day, which corresponds to the endogenous production rate under normal conditions. As a rule, an anti-inflammatory effect can be achieved only with doses of over 75 mg of cortisol per day in adults and 45 mg/m^2 in children.

Wound healing is closely related to inflammatory processes and is also inhibited by the anti-inflammatory action of cortisol. In particular, cortisol impairs the formation of granulation tissue, but hardly affects epithelization. In practice, however, wound healing is not much affected in patients treated with high doses of cortisol. Healing of the operation wound following adrenalectomy is not much delayed, even with daily doses of 50 to 100 mg of cortisol.

SELYE contrasted the anti-inflammatory action of glucocorticoids with the inflammatory effect of mineralocorticoids. Large doses of cortexone promoted inflammatory processes in animals following unilateral nephrectomy or during excessive salt intake. This effect was suppressed by cortisol. However, it is very questionable whether mineralocorticoids play any role in the pathogenesis of inflammatory diseases (see p. 311).

Muscles. Cortisol is necessary for normal muscular activity. Excessive cortisol leads to atrophy and fibrosis due to protein degradation.

Lymphatic Tissue and Thymus. During cortisol therapy, lymphocytes disappear from the lymph nodes and from the thymus. Reticular tissue shrivels. The nuclei become pyknotic, and the reticular network is destroyed. The rarity of intermediate forms between immature and ma-

ture lymphocytes and signs of degeneration of the mother cells indicate that the formation of lymphocytes and thymocytes is impaired. Cortisol also affects the lymphocytes, reducing their cytoplasm, destroying the nuclei, and inhibiting mitosis. Recent investigations on the influence of cortisol on thymocyte metabolism give some insight into the mode of action of glucocorticoids on protein and RNA metabolism, and into the role of these biochemical events in the immunization process. In rat thymocytes exposed to cortisol *in vivo* or *in vitro*, RNA, DNA and protein synthesis are reduced, and the transport of precursors of nucleic acids and proteins across the cell membrane is delayed. This inhibitory effect can only be observed when the medium contains a source of energy. Cortisol injected *in vivo* causes a further reduction in the activity of the DNA-dependent RNA polymerase and thus in ribosomal-protein synthesis (MAKMAN, 1967). All these effects are interdependent. Cortisol also produces signs of degeneration in the mast cells and causes a reduction of their number.

An effect of cortisol on phagocytosis of the reticuloendothelial system is controversial.

7. *Effect on Blood Cells.* Blood cells are influenced by cortisol. Cortisol leads to an increase of total leukocytes, but reduces the numbers of eosinophils and lymphocytes. It increases the number of thrombocytes. The effect on the red blood cells is less distinct: adrenalectomy leads to normochromic, normocytic anemia with increased osmotic resistance of the erythrocytes, which can be prevented by cortisol administration. Whereas polycythemia can be a manifestation of Cushing's syndrome, it has not been observed to result from cortisol administration.

Eosinophils. The eosinophil count is usually in inverse proportion to the cortisol concentration in the plasma, but the individual variations in this parameter are very wide (for diurnal variation see p. 301).

Eosinopenia induced by cortisol is of some diagnostic importance. It can be observed after oral as well as after intravenous administration of cortisol, and reaches a maximum within 4 hours. The resorption of cortisol given by i.m. injection is too slow to provoke a distinct eosinopenic effect. The eosinophils return to the original level as the cortisol concentration falls in the blood. The eosinophil count remains low in the presence of constant high cortisol concentration.

The mechanism of the eosinopenic effect of cortisol is still not clearly understood. The following possibilities have been considered:

1. Inhibition of supply from bone marrow.
2. Fixation and decomposition in specific organs (spleen, lungs, reticuloendothelial system).
3. Direct destruction by cortisol.

Observations on an influence of cortisol on the eosinophils of bone marrow are contradictory. The eosinopenic effect of cortisol is manifest even in the absence of the spleen. An increased number of degenerative forms of eosinophil leukocytes in body fluids has been observed during cortisol administration. However, extensive investigations have excluded a direct effect of cortisone and cortisol on the eosinophils. The eosinopenic action of cortisol is abolished when the reticuloendothelial system is blocked. *Cortisol appears to sensitize the eosinophils to destructive forces independent of the adrenals and also to inhibit the efflux of the eosinophils from organs in which they are formed, stored, or degraded.* Cortisol may attack the enzyme systems of nucleic acid metabolism. For mast cells see p. 305.

Lymphocytes. In contrast to the eosinophil count, the lymphocyte count returns to normal after a short period of time in spite of continued cortisol administration. It is uncertain whether cortisol destroys lymphocytes or whether the lymphopenia is due to the inhibition of efflux from the reticuloendothelial system. The lymphopenic action affects the lymphocytes in the blood-stream as well as the fixed lymphocytes of the lymphatic tissue and the thymus.

Neutrophil Leukocytes. The neutrophil leukocyte count increases under the effect of cortisol. This is due to stimulation of the bone marrow, and long-term cortisol therapy can lead to myeloid hyperplasia. Fatty infiltration of the bone marrow, with increased erythroblasts and decreased myeloblasts, has also been observed during cortisol therapy.

Thrombocytes. The thrombocyte count is increased by 30 to 60% within 6 hours after cortisol administration. The other clotting factors are not influenced.

8. *Effect on Circulation.* Cortisol is probably involved in blood pressure regulation, for hypotension is a sign of Addison's disease and hypertension of Cushing's syndrome. However, whereas cortisol can restore normal blood pressure in adrenal failure, hypertension cannot be induced in healthy subjects even by long-term administration of cortisol in high doses unless they have a predisposition. Cortisol

does not affect plasma renin and angiotensin levels. On the other hand, there is a synergism between noradrenaline and cortisol. Cortisol has a permissive effect on noradrenaline action. Noradrenaline raises blood pressure only in the presence of cortisol. Deoxycorticosterone is ineffective. Cortisol can lead to morphological alterations of the blood vessels. In experimental animals it induces vascular reactions similar to those observed in the Kimmelstiel-Wilson syndrome in diabetics. In man, changes reminiscent of diabetic retinopathy have been observed during cortisol administration. Capillary resistance increases, but increased fragility of the large vessels leads to suffusions: this is frequently observed in Cushing's syndrome.

There seems to be some connection between the action of cortisol on vessels and arteriosclerosis. Serum cholesterol is not changed by cortisol. Nevertheless, arteriosclerosis has been observed in 11-year-old children after long-term administration of cortisone in high doses. On the other hand, cortisol apparently has no influence on the S_f 10-20-lipoproteins, which are related to arteriosclerosis.

9. Effects on the Gastrointestinal Tract. Cortisol promotes the production of hydrochloric acid and pepsin by the stomach, but not regularly. The increased excretion of uropepsin is due to increased clearance. Neither the first vagal phase nor the second phase of hydrochloric acid secretion is mediated by increased cortisol secretion. On the other hand, cortisol appears to stimulate the sensitivity of the gastric mucosa to histamine and to increase the number and height of its parietal cells. Gastric ulcers occur more frequently during high-dose cortisol therapy. They are rarely found in untreated Addisonian patients, but are not more frequent in Cushing patients than in normals. Even achylia does not prevent the development of cortisol-induced ulcers. The pathogenesis is uncertain. Cortisol appears to alter the composition of the gastric mucus, thus affecting its protective action on the gastric mucosa (SPIRO, 1960).

10. Effects on the Nervous System. In animals, high doses of cortisol can lead to chromatolysis and cytoplasmatic vacuolization in the regions of the hypothalamic paraventricular and supraoptic nuclei.

Retardations in the electroencephalogram of Addisonian patients disappear during cortisone maintenance therapy. In Cushing's syndrome and after treatment with high doses of cortisone, abnormalities not due to changes in glucose metabolism are observed in the electroencephalogram. Cortisol increases the excitability of the brain. This is seen in the lowered threshold for the induction of generalized convulsions by an electric shock. Deoxycorticosterone has the opposite effect.

Cortisol therapy can lead to the development of an endocrine psychosyndrome characterized by chronic changes in mood and motivation, excessively uncertain temper, and sudden compulsive urges, which may develop into a short-term psychosis.

The stimulation of appetite by cortisol is possibly due to increased carbohydrate turnover.

11. Effect on Pregnancy. In rats but not in monkeys, malformation of the fetus has been observed after treatment with high doses of cortisol during pregnancy. In humans, fetal damage has not yet been observed following treatment with cortisone or ACTH in normal anti-inflammatory doses during pregnancy. Intensive treatment during the last month of pregnancy may result in temporary adrenal insufficiency of the newborn (hypoglycemic coma).

12. Correlation with Other Endocrine Glands. Cortisol inhibits TSH secretion at a suprahypophyseal level (WILBER, 1969). For correlation with catecholamines, see Chap. VIII, p. 424.

13. Effects on the Whole Organism. Cortisol plays a significant role in the defense mechanism of the body and in nonspecific as well as specific resistance. The lack of resistance in patients with adrenal failure has been known since ADDISON's classic description. The classic bioassay for adrenocortical hormones is based on increased tolerance of poisons in adrenalectomized animals.

Little is known about the mechanism of this striking increase in nonspecific resistance under the influence of cortisol. Its metabolic effects may play a role, in particular the rapid availability of glucose and the influence on enzyme systems. See also general adaptation syndrome, p. 310.

On the one hand, the effect of cortisol on specific resistance, i.e. immunity, is related to its anti-inflammatory effect. The anti-inflammatory action (p. 305) is not restricted to inhibition of local inflammatory processes, but also pertains to general reactions, such as fever and other general toxic manifestations. However, resistance is not always increased by these anti-inflammatory effects, but may also be diminished, because the impairment

of an inflammatory reaction can be harmful and promote the propagation of infectious agents (BEISEL, 1969).

On the other hand, cortisol may inhibit the formation of antibodies when it is administered prior to the antigen, possibly through inhibition of protein synthesis or by some effect on the antigenic stimulus. This effect is probably related to the involution of the thymolymphatic apparatus. Cortisol may enhance the transport of antibodies into the intracellular space.

Cortisol suppresses allergic reactions by altering the reactivity of the tissues. The antigen-antibody reaction itself cannot be inhibited. It affects the formation of histamine, but not the action of formed histamine. Cortisol impairs the conversion of kininogen to kinin by kallikrein (see Chap. XV).

γ) Cortisone

Cortisone has been isolated from the adrenal cortex, but is found only in traces in adrenal venous blood and in peripheral blood. Cortisol is partly converted to cortisone before it is inactivated by the liver. Conversely, cortisone can also be converted to cortisol. In principle, cortisone has the same physiological activities as cortisol, but is approximately 25 to 50% less active. In contrast to cortisol, cortisone has no anti-inflammatory effect when applied locally.

δ) Synthetic Corticosteroids

The introduction of halogen atoms or methyl groups and the reduction with formation of new double bonds lead to qualitative and quantitative changes in the activities of cortisol, thereby increasing the desired therapeutic properties and diminishing or abolishing undesirable side-effects.

Dissociation of the various activities is possible in principle. Thus, the sodium-retaining activity, unwanted in anti-inflammatory therapy, has been successfully removed. On the other hand, the anti-inflammatory effect has not yet been dissociated from the harmful catabolic and gluconeogenetic activities, which are always in proportion to the therapeutic effect. Also, ACTH suppression always parallels the anti-inflammatory activity in all compounds used in therapy, although some corticosteroids are known which actively suppress ACTH secretion but have only minimal effects on the eosinophils and on glucose metabolism (KENDALL, 1963).

The following alterations of the cortisol molecule are of therapeutic importance:

– Reduction of the A ring (double bond between C-1 and C-2) increases glucocorticoid activity and decreases mineralocorticoid activity.

– Fluorination in position 9α greatly increases the glucocorticoid activity, but enhances the mineralocorticoid activity even more strikingly.

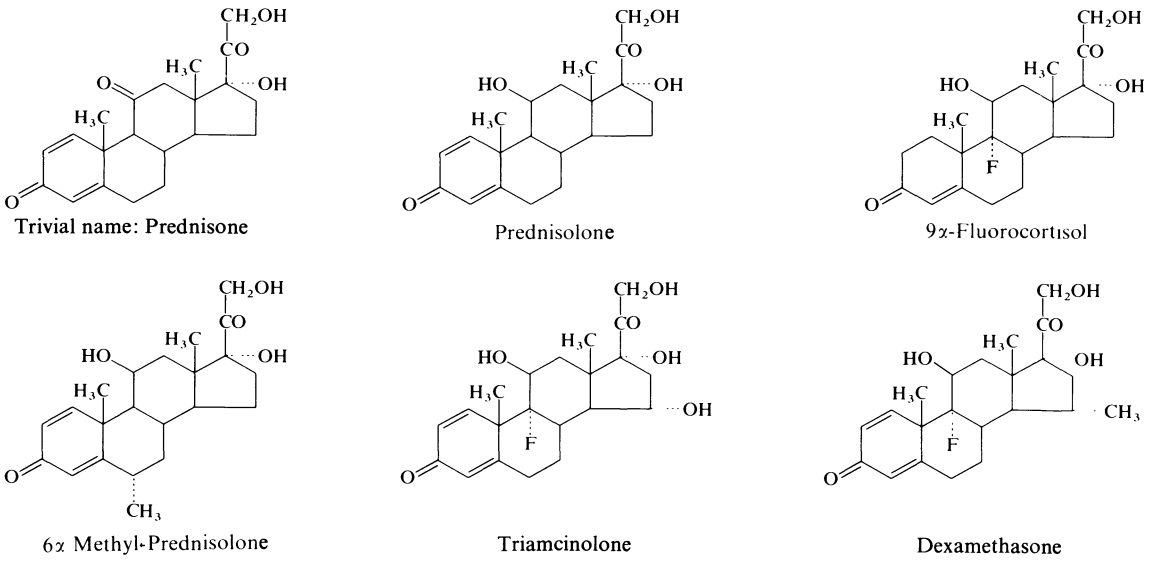


Fig. 13. Structures of the most important synthetic corticosteroids

– Methylation in 6α increases glucocorticoid activity.
– Methylation or hydroxylation in 16α decreases the mineralocorticoid activity.

The following synthetic corticosteroids are of therapeutic importance:

Prednisone, Prednisolone. These are the two synthetic steroids most frequently used today. Their glucocorticoid activity is 4 to 5 times greater than that of cortisol, whereas their mineralocorticoid activity is much lower. Even when given in high anti-inflammatory doses their sodium- and water-retaining effect is negligible. Their other side-effects, such as hyperglycemia, negative nitrogen balance leading to osteoporosis, peptic ulcers, rounding of the face, and acne, parallel their increased therapeutic effect.

Dexamethasone. Among the corticosteroids used in therapy, dexamethasone has the highest anti-inflammatory, hyperglycemic and ACTH-suppressive activity per mg. Sodium-retaining activity is completely absent. Since its metabolites in the urine are quantitatively negligible, this compound is useful for the diagnostic dexamethasone-suppression test (see p. 389), as well as for suppressive therapy in which the excretion of urinary steroids is used as a parameter of pituitary activity.

Table 3. Steroids with equivalent antiphlogistic activity

Cortisone	25 mg
Cortisol (hydrocortisone)	20 mg
Prednisone	5 mg
Prednisolone	5 mg
Methyl-prednisolone	4 mg
Triamcinolone	4 mg
Paramethasone	2 mg
Dexamethasone	0.75 mg
Betamethasone	0.75 mg
Water-soluble preparations:	
Cortisol-hemisuccinate	
Prednisolone-hemisuccinate	
Prednisolone-Na-tetrahydrophthalate	
Methyl-prednisolone-hemisuccinate	
Dexamethasone-phosphate	

The glucocorticoid activities of other synthetic corticosteroids, such as triamcinolone, methyl-prednisolone, paramethasone, and betamethasone, are between those of prednisone and those of dexamethasone. These compounds offer no advantages. They are characterized by differences in protein binding and in biologic half-life. It is not known whether this is of therapeutic importance (see Chap. XX).

Fluorocortisol. 9α-Fluorocortisol has a high glucocorticoid activity but an even more potent mineralocorticoid activity, equal to that of aldosterone. It can only be used locally as an anti-inflammatory agent, because of its potent sodium-retaining activity.

However, it is fully active as a mineralocorticoid when given orally and is therefore very useful as a supplement to cortisone in maintenance therapy of adrenal failure (see p. 329).

Table 4. Effects of adrenocortical hormones

Mineralocorticoids:	Sodium retention
	K^+-, H^+-, Mg^{++}-, NH_4^+-excretion
	Expansion of extracellular space
Glucocorticoids:	Increase of glucose turnover
	Elevation of blood sugar
	Promotion of gluconeogenesis
	Diminution of glucose tolerance
	Catabolic activity
	Promotion of glomerular filtration
	Eosinopenic activity
	Lympholytic activity
	Antiphlogistic activity
	Immunosuppressive activity
Androgens:	Pubic and axillary hair of the woman
	Excessive amounts:
	Acne
	Anabolic and virilizing activity

c) Adrenal Androgens

Little is known about the physiological importance of the secretion of adrenal androgens (dehydroepiandrosterone sulfate, androstenedione, 11β-hydroxyandrostenedione), which is considerable both in man and in woman. The anabolic effect of these predominantly weak androgens becomes important only when they are secreted in excessive amounts. ALBRIGHT's term "N-hormone" (nitrogen-retaining hormone) is not applicable to these androgens. It is still uncertain whether dehydroepiandrosterone, which, strangely, is secreted mainly as a sulfate, has any biological function at all or is merely a by-product of corticosteroid synthesis. The concentration of this compound in the plasma is almost as high in the newborn as in adults, but rapidly decreases to levels too low for assay after birth. It reappears in both boys and girls at the time of puberty, reaches a maximum between the ages of 20 and 40, disappears again in advanced age. The diurnal variation parallels that of cortisol. It is possible that dehydroepiandrosterone first promotes the spurt of growth during puberty in girls (see Chap. XIX) and then terminates it.

The only definite physiological effects of the adrenal androgens are the production and maintenance of pubic and axillary hair, and the stimulation of the sebaceous glands in the woman. The assumption that the psychosexuality of the woman is dependent on adrenal androgens is only partially true. The isolated loss of these hormones does not always affect the sexuality of the mature woman. The individual life history appears to be more important. Androgens are less important than estrogens. They are not important to the psychological maturation of girls (BLEULER, 1964).

Recently, the possibility has been considered that certain metabolites of steroid hormones may be of physiological or pathological importance. Thus, it is known that androsterone lowers serum cholesterol. Steroid metabolites can be active as coenzymes of various dehydrogenases. New possibilities have been suggested by the discovery that etiocholanolone and other steroid metabolites with 5β-H configuration (pregnanolone, pregnanediol, 11-ketopregnanolone, lithocholic acid) can produce fever in man. Intramuscular injection leads to a local inflammation, but not to an increased plasma concentration of this steroid. In contrast, elevated levels of plasma etiocholanolone are found during the periodic attacks of fever in patients with so-called etiocholanolone fever. This is probably due to an impairment of androgen metabolism and conjugation of genetic origin (see Chap. IX, p. 449). Moreover, C_{19}- and C_{21}-steroids and their metabolites, such as etiocholanolone and pregnanolone, can promote the synthesis of porphyrin (KAPPAS, 1968).

4. The General Adaptation Syndrome

According to SELYE'S theory of the general adaptation syndrome, various stresses, such as cold, heat, exhaustion, hunger, infection, intoxication and somatic and psychic trauma, lead to specific alterations in the organism, depending on the type of stress, and to a stereotype response of the organism in the form of a clearly defined syndrome. The most striking manifestations of this general adaptation syndrome are involution of the thymolymphatic apparatus, eosinopenia, gastroduodenal ulcers, hyperplasia of the adrenal cortex with depletion of lipids and cholesterol, and increased excretion of corticosteroids in the urine.

A major role within this mechanism of adaptation is played by the pituitary adrenocortical system, as is suggested by the fact that several important manifestations of the syndrome, i.e. eosinopenia, involution of the lymphatic system and increased secretion of adrenocortical hormones, are not observed after hypophysectomy or adrenalectomy. The development of gastro-duodenal ulcers is not dependent on the integrity of the pituitary adrenal system. Their pathogenesis is not known.

The course of the general adaptation syndrome is characterized by three distinct phases: During the first phase, the so-called alarm reaction, adaptation is not yet developed. This stage is characterized by shock, hemoconcentration and increased capillary permeability. In response to shock, counterdirectional changes occur. The second stage, increase in resistance, is characterized by optimal adaptation. The third phase is the stage of exhaustion, when adaptation is lost again.

The mechanism of adaptation is as follows: A damaged area of the organism informs the pituitary adrenal system through a primary mediator of unknown nature. The pituitary reacts by releasing ACTH, which activates the adrenals.

According to SELYE, the adaptation syndrome can be beneficial or harmful to the organism, depending on the conditions. During the phase of resistance, the defense against other noxious influences may be either increased or decreased. SELYE called the first process crossed resistance and the second crossed sensitization. Thus, even in the stage of resistance, the defense against all nocuous influences is not increased.

This part of SELYE'S theory has been confirmed by clinical experience. It explains why patients with adrenal failure due to Addison's disease or hypopituitarism are defenseless against additional stress and can die in shock during infection, accidents, or surgery.

The theory of the general adaptation syndrome has also solved the problem of the connection between the thymus gland and sudden death. In cases of sudden death, an enlarged thymus gland and strongly developed lymph nodes are generally found. This has led to the term "status thymolymphaticus". The overdeveloped thymus and lymphatic tissue used to be considered a direct cause of sudden death. However, during the First World War it was observed that status thymolymphaticus was also a common finding in youths dying from war injuries. Thus, the pronounced development of the thymus and the lymphatic system is normal in youths, whereas the atrophy of these organs found at autopsy is the result of prolonged illness.

The overactivity of the pituitary adrenocortical system during the stage of resistance evoked by stress leads to shrinkage of lym-

phatic and thymus tissue. It is uncertain to what extent the status thymolymphaticus may also be due to insufficiency of the pituitary adrenal system. It is possible that individuals with status thymolymphaticus cannot respond adequately to stress with an increase in the activity of the pituitary adrenal cortical system.

Clinical investigation of the reaction to stress by the human organism with an intact pituitary adrenal cortical system has largely confirmed SELYE's experimental findings. Both muscular work and hypoglycemia lead to an increase in plasma corticosteroids. Surgery can cause a rise in plasma corticosteroids, but so can anesthesia alone, or even the strain of the preoperative day. Psychological strain in soldiers awaiting a battle, or the psychological excitement of sport contests rather than the physical exertion, lead to increased cortisol secretion. A submaximal rise in plasma corticotropin accounts for the rise in plasma cortisol during surgical operations.

In particular, acutely developing illnesses accompanied by pain cause a rapid rise of plasma cortisol, which is due rather to the pain than to the organic lesion. Analgesic treatment brings the plasma corticoids back to normal. An elevation in plasma cortisol is characteristic in myocardial infarction, pancreatitis, aortic aneurysm, pulmonary edema, renal infarction and diabetic coma. However, it is not pronounced in chronic febrile conditions where there is septicemia but no acute complications. In other diseases, an increase in plasma cortisol may be entirely absent despite an intact pituitary adrenal system, according to a normal ACTH test. Finally, the extreme agonal increase in plasma cortisol immediately before death is not due to a final stimulation of the extremely overactive adrenal cortex, but rather to insufficient catabolism by the damaged liver. Similarly, a postoperative rise in plasma cortisol is due partly to increased secretion, but partly also to delayed degradation and inactivation.

Whereas it is true that the pituitary adrenal system is activated by stress, the usefulness of this reaction and the possible therapeutic value of cortisol and ACTH in this situation is still debatable.

First, there is no correlation between the extent of adrenal stimulation and the severity of the stress or the damage to the organism. The fact that the degree of adrenal reaction is dependent on the subjective effects of stress, in particular pain and fright, rather than on physical damage leads one to doubt the usefulness of this reaction in the defense of the body against stress. Recovery from surgery is not dependent on the level of plasma cortisol. Patients with moderately increased plasma cortisol can recover rapidly, whereas even a high plasma cortisol cannot prevent a fatal outcome. A slight increase in plasma steroids does not indicate an unfavorable course of the illness. It is therefore not reasonable to stimulate the system further by ACTH or to administer cortisol. An intact pituitary adrenal system is always capable of producing the cortisol needed for the defense reaction. In some cases, suppression of this system may be preferable (hypothermia, anabolic hormones).

Finally, experiments have shown that cortisol plays only a permissive or conditioning role in the metabolic events following stress. As demonstrated by INGLE, the negative nitrogen balance induced by stress depends on the presence of cortisol, but is quantitatively related only to the extent of damage and not to the amount of cortisol present.

Administration of cortisol to improve nonspecific resistance is only indicated in established pituitary or adrenal insufficiency. This statement does not apply to the pharmacological use of cortisone and prednisone as anti-inflammatory drugs when this is clearly indicated (see Chap. XX).

Besides describing this specific manner of reaction of the organism, SELYE has also attempted to explain a number of diseases (polyarthritis, periarteritis nodosa, nephrosclerosis, myocarditis) on the basis of the general adaptation syndrome by assuming overshooting, i.e. a harmful reaction of the organism in response to noxious agents. He proposed that the mineralocorticoids promoted inflammation while the glucocorticoids had anti-inflammatory properties. The anti-inflammatory hormones would be stimulated by ACTH, the pro-inflammatory hormones by somatotropin or by an adrenoglomerulotropic hormone. The diseases of adaptation would thus result from a dysfunction of the adaptation syndrome, i.e. from either too much or too little of the pro- or anti-inflammatory hormones, or from a shift in their ratio. Finally, the responsiveness of the end-organs might be altered by the influence of conditioning factors, thus leading to pathological reactions. Although SELYE has tried to support his theories by extensive experimental evidence, they are not consistent with clinical experience. Experimental induction of inflammation by pro-inflammatory hormones is not possible in intact animals, but only in animals subjected to partial nephrectomy or salt loading. Although these experimental changes are morphologically similar to diseases of adaptation, they cannot be compared to human diseases. Moreover, it can be argued that chronic stress

does not lead to diseases of adaptation in the human.

Since the beginning of this century, the problem of defense has fascinated the most eminent physiologists and clinicians. CANNON, HESS, and HOFF have centered their studies on the role of the autonomic nervous system. A first ergotropic phase aimed at immediate action and dominated by the sympathetic nervous system is followed by a trophotropic, parasympathetic recovery phase aimed at restitution. The nervous system plays a central role in the regulation of this mechanism of defense.

SELYE deserves the credit for recognizing and emphasizing the importance of the endocrine system, and in particular that of the pituitary adrenal system, in defense. There are striking parallels between SELYE's model and that of CANNON, HESS, and HOFF. Accoding to both theories, the organism switches its metabolism from construction to performance in the interest of rapid action; in the emergency reaction this is achieved by secretion of adrenaline and increased activity of the sympathetic nervous system, and in the general adaptation syndrome by secretion of glucocorticoids which shunt away the available substrates from protein synthesis to glucose production. In this situation, adrenal medulla and cortex serve the same purpose, with adrenaline and the sympathetic nervous system responding immediately and the adrenal cortex coming into action when stress is prolonged and metabolism must be readjusted. The theory of the general adaptation syndrome has had a very stimulating effect on modern medicine, since it emphasizes the reactivity of the organism in illness. The primary noxious agent is not as important as the way in which the organism reacts to it. SELYE has brought back the "terrain" to the center of attention.

E. Adrenocortical Insufficiency

1. Classification

As an organ of central importance in homeostasis, the adrenal gland itself has a high capacity for adaptation to the needs of the organism. Its size depends on the degree of stimulation by the pituitary gland (see p. 296). A manifold increase in its volume can be observed after chronic hyperfunction; inactivity of the pituitary gland leads to reversible atrophy of the organ. Even fragments of normal adrenals can maintain an adequate hormone production. Overt symptoms of chronic insufficiency can only be observed when over 90% of the organ has been destroyed. The adrenal gland has a very high capacity for regeneration. Extensive resection is known to have been followed by considerable regeneration during ACTH stimulation.

The term adrenocortical insufficiency includes all conditions in which hormone production does not meet hormone requirements, whether these are due to functional disorders of the pituitary adrenal system or to destruction of the organ. The differentiation between primary and secondary adrenal insufficiency is of major theoretic and therapeutic importance.

Primary adrenocortical insufficiency is caused by damage of the adrenal cortex itself. In *secondary* insufficiency, the stimulation by the pituitary gland is insufficient. The adrenal cortex itself is intact, but may be atrophic.

Acute adrenocortical insufficiency, or Addisonian crisis, is a special illness and necessitates special treatment. It can arise from primary as well as from secondary chronic adrenal insufficiency.

The term "relative adrenal insufficiency" does not define a clinical entity and should be avoided (see p. 327).

2. Primary Chronic Adrenal Cortical Insufficiency, Addison's Disease

The disease of adrenal failure described by ADDISON in 1856 is characterized by a slow onset and a chronic course. Acute exacerbations and crises, however, may occur at any time during the course of the disease. Not infrequently, it is the acute crisis which leads to the diagnosis. Classic Addison's disease, however, is typically a chronic illness.

a) Incidence

Addison's disease is rare. In five years, this diagnosis was made in only 8 of 66841 patients attending the Medical Outpatients' Clinic of Zurich University Hospital. Between 1958 and 1963, there were only 31 cases of Addison's disease among 17620 hospital admissions to the Department of Medicine. According to foreign statistics, the incidence of Addison's disease is 1 per 4000–6000 hospital admissions. General statistics of the cause of death, which are not very reliable, show one case of Addison's disease in 250000 deaths. In the 5 years from 1948 to 1952, 12 cases of Addison's disease were observed among 8413 autopsies carried out by the Department of Pathology of the Zurich University Hospital. In an epidemiological study, the morbidity of the disease among the adult population of England was estimated to be 0.04‰ (MASON, 1968).

The incidence of the disease appears to be increasing slightly. However, this is probably only an apparent increase due to improved diagnostic methods. Some statistics show a predominance of male over female patients in a ratio of 2 : 1 (PASCHKIS, 1967; THORN, 1951). According to other authors, the disease is equally distributed between the two sexes (SOFFER, 1961). The age group from 30 to 50 is particularly prone to the disease. The disease is uncommon in the very aged and in children under 15. Combined statistics of Zurich and Baltimore give an incidence of only 2 cases of Addison's disease in 143000 patients under 15. An increased familial incidence of the disease is typical for primary adrenal atrophy and related syndromes (see below). The fact that certain races (negroes, Spaniards) are particularly affected may be related to the higher incidence of tuberculosis in these populations.

b) Etiology, Pathogenesis, and Pathologic Anatomy

Addison's disease is caused by destruction of the adrenal cortex. As stated on p. 312, at least 90% of the cortex must be destroyed before the typical signs of insufficiency become apparent.

Among the causes of Addison's disease, tuberculosis seems to be decreasing. The ratio of tuberculous to non-tuberculous destruction of the adrenal cortex is shifting in favor of the latter form. GUTTMAN (1930) found an incidence of tuberculosis of nearly 70% among the 566 cases he observed personally or reviewed from the literature. In Switzerland it is still the most frequent cause of Addison's disease.

Primary or cytotoxic idiopathic adrenocortical atrophy, whose etiology had been completely unexplained until recently, is now increasing in importance. In Switzerland it is not uncommon, but has not reached the relative frequency found in the United States, where FRIEDMAN (1948) observed 10 cases of tuberculous Addison's disease and 15 cases of primary adrenocortical atrophy in a study carried out at the Armed Forces Institute of Phatology from 1941 to 1946. According to a more recent epidemiological study, the incidence of tuberculous Addison's disease is 0.012‰ and that of primary atrophy is 0.027‰ among the adult population of England (MASON, 1968).

Other bilateral infections of the adrenal cortex, degenerative and inflammatory processes, such as mycosis (blastomycosis, histoplasmosis, coccidiomycosis) and tumor metastases are of minor importance as causes of Addison's disease (less than 10%, GUTTMAN, 1930).

α) So-Called Primary, Cytotoxic, or Idiopathic Adrenocortical Atrophy ("Immune Adrenalitis")

Radiological evidence of adrenal calcification is never found in so-called primary adrenal cortical atrophy. Autopsy reveals no evidence of caseation, no signs of specific inflammation, such as tuberculosis or histoplasmosis, or of other rare causes of adrenocortical destruction such as hemorrhage or tumor metastases, but extensive lymphocytic infiltration with severe atrophy of the adrenal cortex. Occasionally, the adrenals are enlarged with striking lymphocytic infiltration. Whereas the female: male ratio for the incidence of tuberculous Addison's disease is given variously as 1 : 1 or 0.6 : 1, the incidence of primary adrenal atrophy is quite definitely higher in women, with a sex ratio of 2.5 : 1. Although primary adrenocortical atrophy can occur at any age, it is more often found in younger patients than tuberculous Addison's disease and is not uncommon before the age of 20. Familial occurrence is common in primary adrenal atrophy, but rare in tuberculous Addison's disease.

Circulating organ-specific antibodies to microsomes and mitochondria of human adrenals are found in over 50% of patients with primary adrenocortical atrophy (leading article, 1967), and in 88% of female patients. In tuberculous Addison's disease, some investigators have never found antibodies, and others only in a few instances. These antibodies are organ-specific but not species-specific and can be determined either with the relatively insensitive immunofluorescence method according to COONS or with the complement fixation test (ANDERSON, 1967; BLIZZARD, 1967; IRVINE, 1967). Consequently the term "autoimmune adrenalitis" is currently used for primary adrenal atrophy. Some investigators have succeeded in inducing inflammatory lesions of the adrenals in guinea pigs by injecting homologous or autologous adrenal tissue together with FREUND's adjuvant. However, it is occasionally possible to induce lesions of this kind by means of the adjuvant alone (ANDERSON, 1967; IRVINE, 1967).

The majority of patients with primary adrenal atrophy or autoimmune adrenalitis prove to have besides the antibodies to adrenal tissue also antibodies to thyroid, gastric mucosa and intrinsic factor; antibodies to the parathyroids are also occasionally found (see Chap. XVIII). However, antibodies to components of the cell nuclei, which are typical of the so-called collagen diseases, are only rarely found in Addison's disease. These diseases also are not more frequent in Addisonian patients. The reader

is referred to Chap. XVIII for a discussion of the hypothetical pathogenesis of autoimmune polyendocrinopathy.

β) Adrenal Tuberculosis

The adrenals may be greatly enlarged and extensively caseated. Occasionally, the caseation may extend to the surrounding adipose tissue. However, very shrivelled, fibrous and scarred organs may also be found. Cortical remnants with hyperplastic areas are often detectable only by microscopy. The medulla is usually completely destroyed. Extensive calcification such as would be detectable by X-rays is rarely present, and was found at autopsy in only three out of 32 cases by GSELL and UEHLINGER (1933). The adrenals are practically always infected by way of the bloodstream from a primary tuberculous complex or a postprimary tuberculous focus, except in the very rare placental infections with fatal outcome during early infancy. Since adrenal tuberculosis progresses very slowly and becomes manifest only after destruction of 90% of the adrenal cortex, the time lapse between tuberculous dissemination into the adrenals and the development of overt adrenal insufficiency, i.e. Addison's disease, may be many years; according to GSELL and UEHLINGER between 7 and 27 years. Tuberculous Addison's disease is therefore a typically adult disease. In one third of the cases examined by GSELL and UEHLINGER, the adrenal tuberculosis was the only active focus. In a further third, Addison's disease was associated with urogenital tuberculosis, and in a quarter with tuberculosis of bones and joints. Secondary miliary tuberculosis originating in the adrenals is only seldom observed, in older patients with poor resistance. Pulmonary tuberculosis has a benign course in Addison's disease, whereas tuberculosis of extrapulmonary organs remains progressive.

γ) Other Inflammatory or Parasitic Diseases of the Adrenals

All other types of inflammation which can lead to adrenal failure are of minor importance against tuberculosis, at least in Switzerland (see p. 313). However, extensive destruction of the adrenals, and thus Addison's disease, may be due to fungal infections, such as histoplasmosis, coccidiomycosis, and blastomycosis, or to parasites such as Echinococcus alveolaris, which is not uncommon. Syphilis often spreads to the adrenals, particularly in the congenital form, and in rare instances may cause fatal adrenal failure in infants.

δ) Changes in Other Organs

In Addison's disease, the number of sparingly granulated mucoid cells in the *pituitary* gland is increased, while there is a decline in the number of densely granulated, markedly basophilic cells. See p. 316 f. for notes on the pigmentation. Hyperplasia of the lymphatic system with enlargement of the thymus is often seen in primary or secondary insufficiency. Apart from generalized cachexia, there are no other significant morphological changes in the organs.

c) Clinical Features

α) History

Although familial occurrence is rare, it has been described in several instances.

Addison's disease begins gradually, and patients are usually unable to say exactly when the illness started.

Fatigue. Fatigue is an obligatory and early symptom. In contrast to neurasthenic tiredness, it is characterized by organic muscular weakness (adynamia) and increased fatigability. Addisonian patients can be efficient in the morning, but they become exhausted towards evening after the day's demands, whereas the neurasthenic is generally aware of fatigue mainly in the morning ("le triste du matin") and improves in efficiency during the day. Adynamia varies in severity from a barely perceptible decrease in muscular efficiency to near paralytic muscular weakness necessitating complete bed rest. Ascending symmetric palsy has been observed. In the advanced stages of the illness, adynamia affects the whole of the musculature. It becomes manifest in the facial expression. Speech is slowed down, the voice becomes weak, and the heart sounds are hardly audible.

Often the fatigue first becomes apparent as an unusually slow convalescence or as a severely weakened state in minor infections or gastrointestinal disorders.

An objective assessment of the degree of muscular weakness can be obtained by means of the dynamometer. Progression and success of treatment can be assessed by repeated measurements. FLEISCH'S bicycle ergometer yields exact data on the muscular function and the fatigability.

The muscular weakness is due partly to an electrolyte disturbance, but mainly to impaired carbohydrate metabolism. An uncommon but impressive symptom, which may dominate the clinical picture, is muscular pain, particularly in the calfs and flanks. In rare instances, spasm

of the flexor muscles of the limbs is observed, particularly in elderly patients, which regresses with cortisone therapy. Paresthesia may also occur. Periodic joint pains without inflammatory signs have also been observed. There are no morphologic changes and the cause is unknown. Muscular wasting is no more severe than in other chronic illnesses.

A Lowered Gustatory Threshold for Salt is demonstrable in most patients not treated with cortisone and is often associated with salt hunger.

Weight Loss. The second obligatory symptom is weight loss. Extreme emaciation is not always found, and there are rare cases of overweight Addisonian patients. But weight loss is always present in the history of untreated patients, and the diagnosis is doubtful if weight increases or remains constant. Weight loss is partially due to dehydration. Addisonian patients drink strikingly little, which is a protective measure against excessive sodium loss. However, weight loss is mainly a consequence of anorexia which varies in severity and may progress to continual nausea and frequent vomiting.

Gastrointestinal Disturbances. Irritation of the gastrointestinal tract is common but not obligatory in advanced stages of the disease. It becomes apparent as weight loss, fat intolerance, nausea, vomiting, constipation and periodic diarrhea, and finally in uncharacteristic abdominal pains, sometimes chronic and sometimes acute, which can lead to a mistaken diagnosis of peptic ulcer, cholecystopathy, or chronic appendicitis. The gastrointestinal disturbances are probably due to the increased secretion of sodium chloride into the intestinal lumen (see p. 302). Reduced production of hydrochloric acid and pepsin by the gastric mucosa, and sometimes histamine-refractory achylia (see Chap. XVIII) may also be involved. Insufficient resorption of fat may also cause weight loss, even without clinically manifest steatorrhea. Cortisone therapy normalizes the fat resorption. Vomiting and diarrhea are dangerous because they can precipitate a crisis by water and sodium loss.

Hypoglycemia. Complaints of numbness, dizziness, or fainting may be due partly to the hypotension (see below), but are often manifestations of hypoglycemia, particularly when accompanied by the typical symptoms of sudden weakness, cold sweat, trembling, hunger, and pallor, and disappear promptly upon administration of rapidly absorbed carbohydrates. These symptoms are generally observed after fasting, in the early morning, or before meals, but occasionally even 1 to 2 hours after meals rich in carbohydrates. Hypoglycemia occurs frequently in gastrointestinal disorders.

The tendency to hypoglycemia is due to the lack of glucocorticoids and disappears upon treatment with cortisone. When glucose is urgently needed, it cannot be made available quickly enough, since gluconeogenesis and glycogen mobilization from the liver are impaired. The blood sugar levels at which symptoms of hypoglycemia become apparent are higher in Addisonian patients (about 60 to 80 mg%) than in healthy subjects (50 mg%). The fasting blood sugar concentration of Addisonian patients is usually in the low normal range. Attacks of hypoglycemia are observed in about half the patients.

Nycturia. Nycturia is a frequent symptom of the impaired diuresis (see p. 305) with larger volumes of urine during the night than during the day. It is the only symptom resulting from the disturbed renal function.

Mental Changes. Mental changes may be noncharacteristic, appearing as mental fatigability, retardation, and inability to concentrate, and can be the main symptoms of the illness. One of our patients with Addison's disease had been treated by a psychologist for inability to concentrate for 6 months before consulting a doctor. According to STOLL and BLEULER, advanced stages of the mental disorder are characterized by persistent personality changes and an amnestic psychosyndrome; in rare instances acute severe psychoses develop. Depressive moods, lack of initiative, and indifference are the main characteristics of the personality changes in untreated patients. However, alterations of mood and drive in the opposite direction, resulting in euphoria, excitement, and tension, are also observed. Sexuality and appetite are often reduced. As in other cerebral diseases, the amnestic syndrome consists mainly of reduced attention and impaired memory, and is caused by disturbed cerebral metabolism. Acute psychoses of the exogenous reaction type (coma, delirium, dazed condition, excitement, confusion, and hallucinations) may occur in Addisonian crisis. Mental changes regress when adequate maintenance therapy is not started too late. Lack of adrenal androgens sometimes has a negative influence on women's psychosexuality. In these cases, temporary androgen substitution therapy may be indicated.

The disturbances of cerebral function can be objectivated by a pathologic electroence-

phalogram. Mental symptoms disappear and electroencephalograms become normal upon treatment with cortisol or cortisone, but not with deoxycorticosterone. Spastic paraplegia has repeatedly been observed (HARRIS-JONES, 1955; PENMAN, 1960).

β) Physical Examination

1. Pigmentation. Pathologic pigmentation of the skin is the most obvious sign, and is rarely absent. When fully developed, it allows diagnosis at first glance. It is caused by an increase of the normal skin pigment, melanin, and its catabolite, melanoid, and differences from normal complexion are only quantitative (Fig. 14).

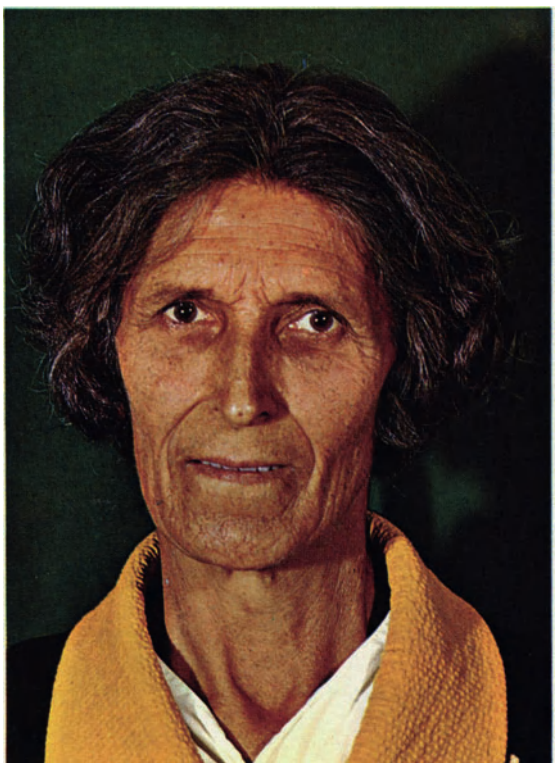


Fig. 14. 59-year-old woman with Addison's disease. Extreme brown pigmentation of the skin, marked weight loss

Slight qualitative changes in the form of an olive gray tinge due to diminished capillary circulation and a relative increase of reduced hemoglobin are, however, possible (Fig. 15). The degree of pigmentation depends more on the patient's individual ability to form pigments than on the duration and severity of the disease. Healthy Mediterranean types may present the same type of pigmentation as Addisonian patients, including the pigmental spots of the buccal mucosa which are often considered to

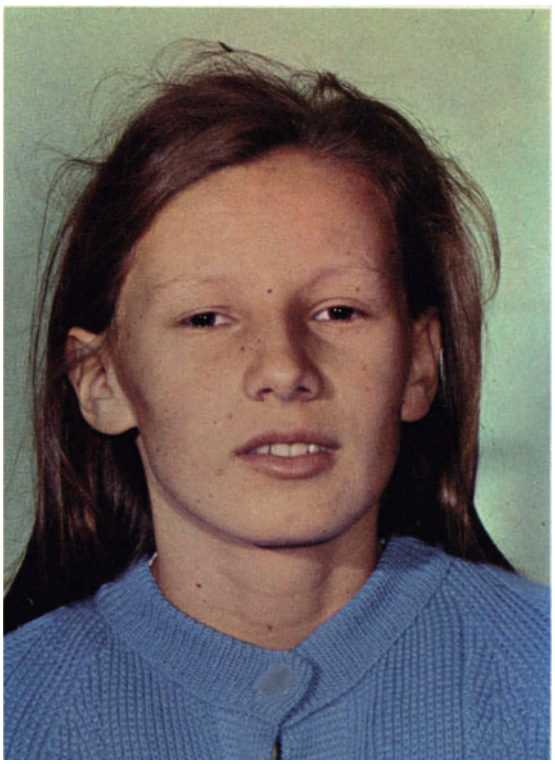


Fig. 15. 15-year-old girl with Addison's disease. Pale, brownish grey complexion, multiple pigmentous nevi in the face and on the neck. Fatigue and severe orthostatic hypotension. After substitution therapy complete remission; the patient became a nurse, married and had two children

be pathognomonic for Addison's disease. On the other hand, increased pigmentation may be completely absent in extremely light-complexioned blond or red-haired patients, or be limited to an increase in the number and size of freckles (white Addison's disease). The decisive factor in the diagnosis is thus not the degree of pigmentation, but an increase which is not explained by exogenous causes. Often, the patients first become aware of the hyperpigmentation when a suntan does not fade as usual after exposure is discontinued.

The increase in pigmentation usually affects the whole body with a predilection for certain areas. These are 1. areas exposed to light, 2. areolas of the nipples, perianal and perigenital regions, 3. skin folds and areas subjected to pressure and friction, and 4. scars acquired after the onset of the illness. Scars which were present before the illness usually remain pale. The dark palmar lines become conspicuous (Fig. 16). The knees, elbows, and knuckles, and areas subjected to pressure from belts and braces take on a dirty grayish-brown color. The characteristic spotty pigmentations of the buccal

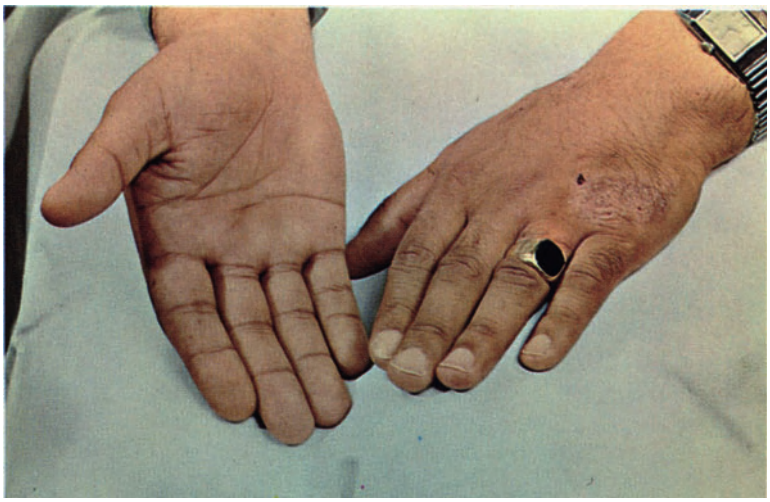


Fig. 16. 35-year-old man with Addison's disease. Pigmentation of the palmar lines and of the finger joints

mucosa can vary from the size of a grain of rice to that of a coin, and from brown to blue-black in color. They are most often found on the inside of the cheeks, frequently on the gums and lips and may also appear on the tongue, the palate, and on the vaginal and rectal mucosa. However, they are never pathognomonic for Addison's disease. Increased pigmentation includes the hair. Darkening of the hair is suggestive of Addison's disease. Besides the diffuse pigmentation, spotty pigmentation in the form of dark brown to black freckles on the face and chest is occasionally observed. Finally, in 10 to 20% of cases, a pigment anomaly appears in the form of vitiligo with irregularly localized areas where pigment is completely absent (Fig. 17).

A perceptible increase in pigmentation appears within three months after total adrenalectomy. However, it may precede the other clinical manifestations of Addison's disease by up to 10 years.

As discussed on p. 295 f., formation of melanin in man is controlled by the melanocyte-stimulating hormone of the anterior pituitary gland (MSH) and ACTH. Addisonian pigmentation is found whenever excessive amounts of ACTH are produced or administered; thus, apart from Addison's disease, after adrenalectomy, in the congenital adrenogenital syndrome and after long-term ACTH administration. Pigmentation decreases in panhypopituitarism and in secondary adrenocortical insufficiency. At present, it is still uncertain to what extent human hyperpigmentation is caused by increased MSH, ACTH, or both (see p. 295).

Pigmentation may be fully developed in latent Addison's disease (see p. 324), because

Fig. 17. 24-year-old patient. Vitiliginous form of skin pigmentation in Addison's disease

the remaining adrenocortical residue is under constant maximum corticotropin stimulation.

Addisonian pigmentation also regresses markedly during long-term treatment with cortisone, which inhibits ACTH secretion. However, only melanin pigmentation is reversible, whereas melanoid pigment remains constant.

The *skin* is otherwise dry and brittle, in contrast to the skin of neurasthenic patients, which is moist and vasolabile. The skin folds remain elevated due to dehydration.

2. Pubic and Axillary Hair. Pubic and axillary hair is sparse in male Addisonian patients. In

women, it is completely absent or is reduced to a minimum. Body hair does not grow again after shaving. Although the absence of pubic or axillary hair in a woman is very suggestive of adrenocortical insufficiency, it may be due to constitutional factors. This is, however, very rare in white in contrast to yellow races.

3. Circulatory Organs. Hypotension: The absence of mineralocorticoids leads to sodium loss and thus to decreased extracellular and intravasal volumes. In addition, the presence of glucocorticoids is necessary for the tonic action of noradrenaline upon the arterioles and the capillaries. Thus, hypotension, a major feature of Addison's disease, has two separate causes. Systolic blood pressure is generally below 80 and 90, and rarely above 110 mm Hg. Diastolic blood pressure is always below 70 mm Hg. In preexisting hypertension, blood pressure is lowered, but may still be above normal.

A loss of vascular tone becomes particularly apparent as orthostatic hypotension. Whereas in healthy subjects a change from a recumbent to a sitting or upright posture induces an increase in blood pressure (measured after 5 min in each posture), it leads to a decrease in Addisonian patients. Addisonian patients frequently complain of dizziness, palpitations, and tachycardia when maintaining an upright posture. A positive orthostatic test is not, however, conclusive, as it can also be a sign of vegetative lability. Treatment with deoxycorticosterone may restore the plasma volume and vascular tone. However, only treatment with cortisol or cortisone can prevent an orthostatic fall in blood pressure.

A decreased plasma volume leads to a decreased stroke volume and a reduction in cardiac output, leading to a small, soft and retarded pulse. The pulse rate increases rapidly on exertion. The size of the heart is related to the intravasal volume and is reduced in untreated Addison's disease. Adequate therapy brings it back to normal.

The ECG of untreated Addisonian patients is characterized either by nonspecific changes in the repolarisation phase, such as flattened or negative T waves, prolongation of the QT, PR, and QRS intervals, depression of the ST segment and low voltage, or signs of hyperkalemia, particularly during Addisonian crisis, such as high, pointed, symmetrical T waves, usually with normal QT intervals. In treated Addison's disease, ECG findings are usually normal.

4. Pathologic Calcification. Calcification of the adrenals can be detected by X-rays in 23% of Addisonian patients. The position is paravertebral at the level of the 1st lumbar vertebra. It can confirm a diagnosis of Addison's disease, but does not exclude an adequate function of the adrenal cortex.

Striking induration of the auricles may be found in Addison's disease of long duration, occasionally even calcification of the cartilage. This is possibly related to the hypocalcemic effect of cortisol.

γ) Laboratory Findings

Anemia. In uncomplicated Addison's disease, anemia is not severe. It is normochromic and normocytic in type, and the hemoglobin is usually between 60 and 75%. It may be masked by hemoconcentration, especially during Addisonian crisis. All hematological investigations should therefore be repeated after rehydration. See Chap. XVIII for pernicious anemia in adrenocortical insufficiency due to primary atrophy. (Pathogenesis is discussed on p. 306.)

Leukocytes. The total leukocyte count is at the lower limit of normal, between 3000 and 4000. The rise during infections is inadequate. There is a relative lymphocytosis, usually of above 35%, which may even be mistaken for leukemia. Infections lead to lymphopenia. The eosinophils are increased, ranging between 100 and 1000/mm^3 with an average value of 300, i.e. 8 to 10% of the total number of leukocytes (normal value 100 to 250 by direct counting, see p. 386). Addison's disease is not likely when the eosinophil count is below 50/mm^3, but can not be excluded. There are constitutional forms of aneosinophilia.

Sedimentation Rate. Sedimentation rate is often increased, even in the absence of infection. Electrophoresis reveals displacement of albumin by globulins, particularly by gamma-globulins.

Serum Electrolytes. Serum electrolytes are only disturbed in advanced stages of the illness. Normal values do not exclude adrenal insufficiency. In severe cases, there is hyponatremia and, to a lesser extent, hypochloremia, both of which may be masked by hemoconcentration. Hyperkalemia is therefore usually better demonstrable. Values of less than 130 mEq/l of sodium (300 mg%) and of more than 5 mEq/l of potassium are suggestive of adrenocortical insufficiency. The sodium/potassium ratio can be altered even when the sodium and potassium values are still within the normal range. A value of below 30 (both electrolytes measured in mEq/l) is suggestive of adrenal insuffiency (normal value 32). Hyper-

calcemia is frequently, but not regularly, found in untreated Addison's disease (DE LORME, 1964; PRADER, 1959). The generally mild, but sometimes considerable hypercalcemia (up to 17 mg%) may be associated with hypercalciuria, thirst, hyposthenuria, polyuria, and normal or elevated serum phosphate values. Intestinal calcium absorption is increased, as is calcium mobilization from the bones. Active calcium excretion through the gut is decreased. The hypercalcemic syndrome disappears with cortisone therapy. According to animal experiments, ionized calcium remains unchanged, whereas protein-bound and complex-bound calcium may be considerably increased.

Urinary, Salivary, and Sweat Electrolytes. Increased sodium excretion in the urine and saliva is even more indicative of adrenocortical insufficiency than a low serum sodium. Whereas urinary excretion is dependent on the dietary sodium intake, estimation of the sodium-potassium ratio in saliva or sweat gives valid information even when the diet is unknown (see p. 391 f.).

Plasma pH. The electrolyte disturbance is paralleled by moderate metabolic acidosis, usually compensated by hyperventilation. It is mainly due to a shift of H-ions from the intracellular into the extracellular space and to deficient ammonium formation and H-ion excretion of the renal tubuli. Severe acidosis is only observed in crisis and in renal failure.

Disorders of Renal Function. The specific renal dysfunction in primary adrenocortical insufficiency consists of three components:

1. Glomerular filtration rate is reduced even when extracellular fluid and plasma volumes are normal. It can only be normalized by cortisol or other glucocorticoids.

2. The patients are unable to produce rapid diuresis. In the healthy subject, a water load of 1 to 1.5 liters is excreted within 4 hours. In adrenal insufficiency, diuresis begins gradually, often during the following night, and the patient is in danger of water intoxication.

Cortisol and other glucocorticoids, but not aldosterone, can normalize diuresis. Deficient diuresis is not due to the reduction of the glomerular filtration rate. Perhaps more antidiuretic hormone is secreted, or its degradation retarded in adrenal insufficiency (AHMED, 1967). Alternatively, cortisol may have a sealing effect on the distal tubular sections and collecting ducts and be necassary for suppression of facultative water reabsorption (KLEEMAN, 1964).

These two types of renal disorder are found in both primary and secondary adrenal failure.

3. Lack of aldosterone leads to loss of sodium and retention of potassium. Finally, during crisis sodium loss leads to dehydration, fall in blood pressure, and shock. Renal circulation decreases and severe renal insufficiency with a rise in blood urea nitrogen develops.

Renal concentrating capacity is only rarely impaired, in contrast to the ability to dilute the urine.

Basal Metabolic Rate. The BMR is usually slightly reduced and varies between -20 and -10%. Thyroid function itself is not impaired, as is indicated by a normal radioiodine uptake and a normal protein-bound plasma iodine. The absence of glucocorticoids, however, leads to retardation of the peripheral metabolic processes.

Carbohydrate Metabolism. Disturbances of carbohydrate metabolism can be recognized both from the history and from stimulation tests (see p. 389). Determination of fasting blood sugar is not very informative.

Urinary Steroids. Determination of 17-ketosteroids, 17-hydroxycorticosteroids, or 17-ketogenic steroids in the urine is highly significant in the diagnosis of adrenal insufficiency. Their failure to respond in the ACTH test is more conclusive than their basal values. Normal values of these three urinary steroid fractions do not exclude adrenal insufficiency, and decreased values may also be due to other factors than adrenal insufficiency.

Plasma Corticosteroids. In advanced Addison's disease, plasma corticosteroids are decreased or too low for assay. However, the values found in Addisonian patients may be within the range of normal diurnal variation. The responsiveness of plasma corticosteroids to ACTH is also more important in the diagnosis of Addison's disease than their absolute values.

Aldosterone. In some cases, determination of the aldosterone excretion or secretion rate can help to differentiate between primary and secondary adrenal insufficiency. Whereas normal aldosterone values are found in secondary adrenal insufficiency, aldosterone is found in the urine of Addisonian patients only in traces, even after salt restriction.

Plasma *renin* is increased in untreated Addisonian patients, even when they are hypotensive; this is probably due to hypovolemia (PEART, 1965).

δ) Test of Adrenocortical Functional Reserve

The laboratory findings mentioned above can be normal in early mild Addison's disease. Tests for assessment of the adrenocortical functional reserve are therefore essential for a definite diagnosis. The ACTH test (Thorn test) is particularly important. This test clearly indicates whether the adrenals are still functioning, to what extent, and how great their functional reserve is. The previously used tolerance tests have been completely abandoned since they are less reliable and more harmful than ACTH tests (see p. 391). It is advisable to treat patients with suspected Addison's disease with dexamethasone, 0.5 mg twice daily to prevent a hypersensitivity reaction to ACTH. However, the eosinophil count is valueless in these conditions. The risk of side-effects can also be reduced by use of a synthetic ACTH preparation.

ε) Review of Diagnostic Procedure

Usually the history, the physical examination including the orthostatic test, and the rapid ACTH test and 48-hour depot ACTH test are sufficient to allow a decision on whether Addison's disease is present or not. If the diagnosis is doubtful, it is advisable to admit the patient to hospital for about 8 days, and weight, pulse rate, and blood pressure must be measured daily during this period. Strong laxatives and morphine are contraindicated. Barbiturates should be given with caution.

Table 5. Frequency of clinical symptoms in Addison's disease. (Based on 94 cases of THORN, 1951)

Weakness and fatigue	100%
Weight loss	100%
Increase in pigmentation	94%
Anorexia	90%
Vomiting	84%
Nausea	81%
Abdominal pains	32%
Obstipation	28%
Diarrhea	21%
Salt hunger	19%
Muscular pains	16%

Table 6. Synopsis of the most important clinical symptoms and laboratory findings in Addison's disease

Clinical symptoms:
Weakness, weight loss, hypotension, increased pigmentation
Laboratory findings:
Pathological results of ACTH-tests, low plasma and urinary corticosteroids, eosinophilia and lymphocytosis, hyponatremia, hypochloremia, hyperkalemia, azotemia, impossibility of quick diuresis

The following examinations are important in the diagnosis of Addison's disease:

1. Hematologic examination: blood smear, sedimentation rate, hematocrit, total protein, sodium, potassium, chloride, urea. Arterial blood: pH, carbon dioxide tension and bicarbonate concentration;
2. Urinary 17-ketosteroids and 17-hydroxycorticoids, plasma cortisol;
3. A two-day 8-hour i.v. ACTH test with determination of urinary steroids, plasma cortisol, or both;
4. X-rays: chest or cardiac teleradiography, abdominal X-ray to reveal adrenal calcification;
5. Renal function tests: creatinine clearance, phenolphthalein excretion;
6. Ergometry, to be repeated after cortisone therapy.

d) Course of Addison's Disease

Untreated Addison's disease has a progressive course over many years. Remission of single symptoms may occur, but the hypotension remains unchanged. Remissions can last for weeks or months. They may be due in part to regeneration of adrenal tissue. More often, the remission is due to an adaptation of the life style to the reduced amounts of hormones produced by an adrenal residue, and thus to a new hormone balance. The disease may thus come to a standstill for many years, persisting as latent Addison's disease with few symptoms (see p. 324). Earlier reports of complete recovery from Addison's disease were probably based on mistaken diagnosis and inadequate laboratory tests. Complete recovery is extremely rare (NORDIN, 1955).

The lack of resistance is characteristic of untreated Addison's disease. The adrenals play a central role in the maintenance of homeostasis (see p. 312). When they are absent, or their functional reserve is exhausted, the organism is incapable of withstanding stress. Physical exertion, trauma, surgery, and infections upset the precariously maintained balance between the production of and the requirement for adrenocortical hormones, leading to collapse and Addisonian crisis. Sudden death may occur at any time without premonitory signs.

Addisonian patients are no more susceptible to infections than healthy subjects, but the course of infections is usually more severe. Allergic diseases often develop at the same time as Addison's disease. The lack of resistance of Addisonian patients is also manifest in intolerance of many drugs, although this may be improved by cortisone treatment. Addisonian

patients are particularly sensitive to narcotics, such as morphine and codeine, and to a lesser extent to sedatives such as bromides and barbiturates. These drugs have to be used carefully and in low doses, particularly when sedatives are needed for restlessness during fever.

e) Pregnancy during Addison's Disease

Important changes in corticosteroid metabolism occur from the second trimester of pregnancy. These are partly due to the increased level of estrogens in the maternal blood. As during therapy with estrogens (see p. 292), the total cortisol concentration in the blood increases. This increase, however, is only due to protein-bound cortisol, whereas the free plasma cortisol remains unaltered. Estrogens also interfere with the enzymatic metabolism of cortisol in the liver. However, whereas estrogen therapy leads to a decreased excretion of urinary steroids, there is a marked increase in the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids in advanced pregnancy, reflecting increased maternal cortisol production (COPE, 1959). The increased urinary levels of aldosterone-18-glucuronide are partly due to the fact that a smaller percentage of secreted aldosterone is converted to tetrahydroaldosterone during pregnancy and more aldosterone is excreted in the form of aldosterone-18-glucuronide. However, the aldosterone secretion rate is also increased, perhaps to compensate the natriuretic effect of progesterone.

NEHER and STARK (1961) have isolated cortisol and cortisone from human placenta, but it is highly uncertain whether the placenta actually releases corticosteroids into the maternal blood. In any case, maintenance therapy cannot be abandoned during pregnancy in an Addisonian patient. Experience has shown that the corticosteroid requirements are increased only immediately before and during delivery.

In the past, pregnancy was rare and hazardous in Addison's disease. Now, however, the incidence of pregnancy is steadily increasing and does not involve any additional risk if adequate maintenance therapy is given.

The daily oral maintenance dose consists of 25 to 50 mg of cortisone acetate and 0.1 mg of fluorocortisol. On the day before and the day after delivery, the daily cortisone dose has to be increased to 200 mg and gradually reduced to the maintenance dose within a week. As a precaution, an intravenous cortisol infusion is started during delivery, as during adrenalectomy. Frequent check-ups are necessary, particularly during the first trimester of pregnancy (weight, edema, blood pressure, blood sugar, electrolytes). The newborn infants of Addisonian mothers weigh 500 g less than normal on average. This is due to the lower maternal blood sugar levels. Pregnancy lasts an average of 13 days longer. Cesarean sections are not usually indicated. There is no cause for anxiety if spontaneous delivery does not occur until two weeks after term. The newborn needs no hormone therapy.

3. Other Forms of Adrenocortical Insufficiency

a) Acute Adrenal Insufficiency, Addisonian Crisis

Any stress increases the organism's requirement for cortisol. If this requirement is not met by increased secretion or administration, disorders with a further stress-producing effect develop, causing a vicious circle in the patient and leading to acute adrenocortical insufficiency. In particular, vomiting and diarrhea can result in massive losses of sodium, chloride and water. Nausea and severe adynamia lasting several days may be the premonitory symptoms of an imminent crisis. However, it can also occur suddenly as a consequence of stress. Acute infectious diseases, physical overexertion combined with salt loss by sweating, and particularly surgery leading to a greatly increased requirement for adrenocortical hormones are dangerous stress situations for Addisonian patients, from which a crisis may develop if appropriate therapy is not instituted. The same applies to secondary insufficiency in panhypopituitarism and to abrupt withdrawal of long-term cortisone administration.

Clinical Features. Addisonian crisis is a very impressive clinical situation. The patient is irritable and restless at first, then becomes increasingly apathetic and finally falls into collapse and coma. There is always a state of extreme weakness, in which even talking is an effort. Dehydration becomes manifest in a dry tongue and dry skin, which can be lifted up in folds. The skin is cold and the body temperature is usually below normal. Development of fever in persisting crisis is a sign of impending death. The systolic blood pressure is below 70 mm Hg, and the diastolic pressure immeasurably low. Pigmentation increases due to dehydration. Circulatory failure adds a cyanotic tint. Vomiting almost always occurs during the crisis. Diarrhea is frequent, and severe abdominal colics, often leading to confusion with acute abdominal processes, may be the predominant feature. Hypoglycemia may cause twitching and choreiform movements. Circulatory collapse leads to renal insufficiency. Azotemia is always present.

Addisonian crisis occurring in patients treated with deoxycorticosterone only in normal or excessive doses is not very different. Dehydration is, however, absent and edema may even be present.

b) "Adrenal Apoplexy": Acute Adrenal Insufficiency due to Hemorrhagic Infarction

Acute adrenal insufficiency due to hemorrhagic infarction occurs in:

1. Newborns;
2. Sepsis, particularly acute meningococcal sepsis, usually in children and rarely in adults (Waterhouse-Friderichsen syndrome);
3. Anticoagulation;
4. Bilateral thrombosis of the adrenal veins;
5. Hemorrhagic diathesis, hypertension.

α) Acute Adrenal Insufficiency in Newborns

The acute adrenal insufficiency of the newborn has been thought to be due to birth trauma. Possibly, however, hemorrhagic diathesis of the newborn (hypoprothrombinemia) is the major factor, and post-natal physiological involution contributes to the susceptibility of the adrenal glands. Adrenal hemorrhage is not uncommon in newborns, and may be considerable. The adrenals are converted into pouches of blood, which are occasionally larger than the kidneys. Most of the infants die within the first days of life. Chances of survival are better in unilateral than in bilateral hemorrhage. A common complication is rupture of hematomas with intra-abdominal hemorrhage. Old hematomas tend to be calcified.

The clinical feature is that of shock in the newborn with tachycardia, irregular pulse, pallor and cyanosis, and cold limbs. Fever and dyspnea may simulate pneumonia, but chest examination is negative.

β) Waterhouse-Friderichsen Syndrome

This syndrome is characterized by the acute onset of lethal sepsis with no noticeable signs of resistance to infection, meningitis, or leukocytosis, with petechial eruptions, and generalized discoloration of the skin reminiscent of postmortem hypostasis, with adrenal hemorrhage ranging from just microscopically detectable to massive bleeding. The patients may be in good health in the morning, fall ill in the evening, and be dead by the following morning. The illness seldom lasts longer than 24 hours. The patients unexpectedly develop high fever and rapidly decline into a state of shock resulting in irreversible circulatory collapse. There are few signs of adrenal insufficiency besides circulatory failure. The eosinophil count is hardly ever increased. Hyponatremia, hyperkalemia, and hypoglycemia are only rarely observed.

As a rule, the adrenals are only slightly enlarged, but they are diffusely or patchily discolored to a deep dark red. They can show all stages of hemorrhagic infiltration, up to complete hemorrhagic infarction of the medulla and cortex. The medulla is necrotic even when the damage is less extensive. Portions of the subcapsular layer of the cortex, however, may still be intact. The cortical tissue shows the features of excessive overactivity with glandular transformations. Cortical necrosis, and particularly hemorrhage of varying extent with inflammatory reactions, are also seen. Thrombi are often seen in the small vessels but rarely in the larger veins.

Pathogenesis. As recognized by BAMATTER in 1935, the illness is almost always due to meningococcal sepsis. The syndrome is only rarely caused by Pfeiffer's bacillus (hemophilus influenzae) or by hemolytic streptococci of group A. The syndrome must be distinguished from meningococcal meningitis, which is associated with leukocytosis and now usually has a good prognosis. According to present theories on the pathogenesis, the disease is due to a process similar to the Sanarelli-Shwartzmann reaction. The vascular endothelia are sensitized by bacterial toxins and react to repeated exposure with intravascular clotting. This leads to hypofibrinogenemia with hemorrhagic diathesis and to infarction.

Originally, treatment with cortisol was recommended in addition to antibiotic and chemotherapeutic therapy, especially when adrenal failure was indicated by an increased eosinophil count as well as by hyperkalemia or hyponatremia. Blocking of intravascular clotting with heparin is now advocated (STUBER, 1961). Cortisol might even be harmful, because it accelerates the Sanarelli-Shwartzmann reaction. Exchange transfusions should be considered. The shock, however, has to be treated with angiotensin II or noradrenaline, and if these agents are not effective, also with cortisol. Until now, no unequivocal case of Waterhouse-Friderichsen's syndrome has been cured, although the prognosis of meningococcal sepsis has improved considerably in this era of chemotherapy and antibiotics.

γ) Adrenal Hemorrhage during Anticoagulant Therapy

Acute adrenal insufficiency caused by adrenal hemorrhage during anticoagulation with di-

coumarol derivatives and particularly with heparin, has been described.

Bilateral adrenal hemorrhage may lead to acute abdominal distress 7 to 10 days after institution of the correct anticoagulation therapy; abdominal pains occur, and meteorism, combined with or immediately followed by muscular defense, fall in blood pressure, pallor, stupor, nausea, vomiting, diarrhea, cyanosis, and shock.

Laboratory findings include leukocytosis with absolute eosinophilia, low serum sodium and chloride, high potassium and blood urea nitrogen, low or unmeasurable plasma and urinary corticoids. The hemorrhagic diathesis may be restricted to the adrenals. Dermatological symptoms, ecchymosis and suffusions are absent.

Immediate substitution therapy can save the patient's life (see p. 330).

Adrenocortical function may recover or may remain permanently damaged.

The pathogenesis is not explained. In some cases, the adrenals may have been previously damaged. An increased secretion of ACTH induced by endogenous or exogenous stimuli may play a role. The time interval between anticoagulation and "adrenal apoplexy" is always 1 to 2 weeks, and the syndrome has not been observed in the further course of long-term anticoagulation.

δ) Thrombosis of the Adrenal Veins

Bilateral adrenal venous thrombosis is rare, and may occur post partum and after severe burns. It has the clinical features of severe shock with very painful abdominal colics and a soft abdomen. Damage to the adrenals may be the primary event in this illness and thrombosis of the veins its consequence.

c) Congenital Chronic Adrenocortical Insufficiency

A. PRADER

The signs and symptoms of congenital chronic adrenocortical insufficiency only rarely appear immediately after birth, usually developing after a few days or weeks. They are nonspecific and consist of vomiting, failure to thrive, weight loss, dehydration, and circulatory failure. After one to three months, an Addisonian pigmentation of the skin can be observed. Beside hemorrhagic infarction of the adrenals (see above), the following three groups of prenatal adrenocortical disorders have to be considered as the cause of this form of adrenocortical failure:

α) Congenital adrenal hypoplasia (see p. 325f.).

β) Congenital adrenocortical hyperplasia (various forms of the congenital adrenogenital syndrome, p. 358ff.).

γ) Congenital *adrenocortical lipoid hyperplasia*. This syndrome, together with the various forms of congenital adrenogenital syndrome, is one of the congenital disorders of steroid biosynthesis with adrenal hyperplasia and is also due to a genetic factor (see Table 16, p. 359). Clinically, this disease is characterized mainly by signs of adrenal insufficiency, appearing at the age of a few weeks (PRADER, 1957), whereas at autopsy the lipoid hyperplasia of the adrenals is the striking feature (SIEBENMANN, 1957). The involvement of the electrolytes is similar to that in Addison's disease, 21-hydroxylase deficiency with salt loss (see p. 369f.) and 3β-hydroxysteroid-dehydrogenase deficiency (see p. 371f.). However, the syndrome differs from 21-hydroxylase deficiency in that the external genitalia are always of the female type, even in patients with a male chromosomal configuration and male gonads. In boys, internal and external genitalia are similar to those in testicular feminization (see p. 726f.). In girls, the genitalia are absolutely normal. As in some other forms of congenital adrenocortical hyperplasia, these children die during the first months of life unless they are treated with cortisol, salt, and perhaps mineralocorticoids (see p. 370). The prognosis is poor, however, even with adequate treatment.

The adrenals are very much enlarged and strikingly yellow. Histology shows extreme adrenocortical hyperplasia with large cells densely filled with lipids. In addition, there are calcium deposits, crystalline lipoid deposits, and multinuclear giant cells. In one case, neither androgens nor estrogens could be found in an extract of adrenocortical tissue. Steroids cannot be detected in the urine of these patients. As in other forms of congenital adrenocortical hyperplasia, the pathogenesis can be explained by an enzyme deficiency leading to a decrease in corticol production. Since no steroids at all are found in these patients, the site of the disorder must be one of the earliest stages of steroid biosynthesis, i.e. one or several of the enzymes involved in the conversion of cholesterol to 5-pregnenolone (Fig. 27, p. 358) must be absent or inactive. Thus, the disease could be due to a deficiency of 20-hydroxylase, 22-hydroxylase, and/or 20,22-desmolase. This enzyme deficiency is not restricted to the adrenal cortex, but also affects the Leydig cells of the testes (PRADER, 1957). This would explain the development of female-type genitalia in boys.

The lack of cortisol leads to adrenocortical failure and to increased ACTH production causing adrenocortical hyperplasia with accumulation of nonutilizable cholesterol, while on the other hand, the absence of androgen production by the fetal adrenals and the fetal testes impedes male differentiation of the genitalia as in 3β-hydroxysteroid-dehydrogenase deficiency (see p. 371 f.) and 17-hydroxylase deficiency (see p. 372).

In girls, it is hardly possible to differentiate lipoid hyperplasia from other forms of congenital adrenocortical insufficiency, such as hemorrhagic infarction of the adrenals, by clinical examination. Differentiation from testicular feminization is possible on the basis of steroid excretion when the patient has male chromosomes. In testicular feminization, the excretion of all steroids is normal for age, whereas in lipoid hyperplasia all steroids are absent. Differentiation from 3β-hydroxysteroid-dehydrogenase deficiency is very simple in both sexes, since in this condition the total 17-ketosteroids, and particularly dehydroepiandrosterone, are elevated. Deficiency of 17-hydroxylase can be excluded in spite of female-type development of the external genitalia, because the electrolyte disorder in these patients is not like that in Addison's disease, but is due to overproduction of deoxycorticosterone (see p. 340) with hypokalemia and hypertension.

d) Latent Addison's Disease (Partial, Potential Addison's Disease, cf. p. 312)

It has long been known that ADDISON'S disease can vary in severity. In view of the slow development of the disease, the patients go through a stage where impaired adrenal function is apparent during abnormal stress before reaching the stage where adrenal failure is manifest under basal conditions.

A latent insufficiency always becomes manifest in situations of stress, such as infection, overexertion, surgery, and trauma. However, the patients are still capable of leading a normal daily life without maintenance therapy. They may feel healthy and even the important symptom of fatigue may be absent or inconspicuous. Symptoms of hypoglycemia are not usually present. Generally, these patients are not aware of their illness as long as they lead a life without undue exertion.

The patients' appearance is not altered except for the skin, which may assume the characteristic pigmentation of Addison's disease at a relatively early stage or merely show increasing numbers of dark brown freckles. Laboratory examinations, however, clearly reveal the insufficient adrenocortical function even at this latent stage of the disease: although the adrenals do function, there is no functional reserve. Urinary 17-ketosteroids and 17-hydroxycorticosteroids may be, but are not necessarily reduced. Plasma corticosteroids are not completely absent, but are in the low normal range. However, neither urinary nor plasma corticosteroids increase under ACTH stimulation. Pathologic results are obtained in any form of ACTH test, including the 8-hour intravenous infusion test repeated over several days: there is no fall in the eosinophil count and no adequate rise in urinary and plasma steroids. Electrolyte disorders are not demonstrable in the blood, saliva, or sweat. These findings indicate that the adrenocortical remnant is capable of producing sufficient amounts of hormones for the basal demands of life only under maximal ACTH stimulation. This explains the pigmentation as well as the pathologic ACTH test. Exogenous ACTH is ineffective because the adrenal cortex is already maximally stimulated by endogenous ACTH.

e) Familial Addison's Disease

There is an increased familial incidence of adrenal insufficiency due to primary atrophy. This disease may be a form of autoimmune polyendocrinopathy (see Chap. XVIII).

Other forms of polyendocrinopathy are Addison's disease associated with hypoparathyroidism, with hypo- or hyperthyroidism, with diabetes mellitus and with pernicious anemia.

f) Addison's Disease with Cerebral Sclerosis

This well-defined syndrome is probably inherited through a recessive gene linked to the X chromosome. So far 10 cases have been observed. The disorder usually becomes noticeable in late childhood (between the ages of 3 and 14) and progresses inevitably to death within months or years. Addison's disease begins with increased pigmentation of the skin and gradually presents all the signs and symptoms typical of *primary* adrenal cortical insufficiency. At about the same time, signs of the neurologic disorder appear. These are ataxia, and myoclonic and choreiform movements, finally progressing to spastic tetraplegia with pyramidal signs, dysarthria and incontinence. The neurologic illness leads to progressive invalidity, the simultaneous organic psychosyndrome to complete dementia. The protein concentration in the cerebrospinal fluid is increased, whereas the cell count is normal.

The pathologic anatomical changes in the adrenals take the form of primary atrophy with few remaining cortical cells. There is no inflammatory infiltration and no scar tissue. The medulla is not altered and the other endocrine organs, particularly the pituitary gland, are intact. There is extensive demyelinization in the cerebellum and the cerebrum; this is sometimes patchy and sometimes diffuse, as in diffuse cerebral sclerosis (Schilder's disease). The pathogenesis is unknown, and simultaneous involvement of two such different organs is puzzling. Antibodies to adrenal tissue have not been found in any of the cases examined. Only the adrenocortical insufficiency can be treated. The brain disease progresses inevitably to death.

g) Selective Deficiency of Single Hormone Groups

Usually all three adrenocortical hormone groups, i.e. the mineralocorticoids, glucocorticoids, and androgens, are deficient in ADDISON'S disease.

Selective absence of *androgens* is a physiologic condition in childhood up to the onset of "adrenarche" between the 8th and 10th years. Isolated complete adrenal androgen deficiency is only observed occasionally in the very aged.

Glucocorticoid and androgen deficiency associated with near-normal aldosterone secretion is typical of secondary adrenocortical insufficiency due to panhypopituitarism, long-term corticosteroid or ACTH therapy, or a cortisol-producing adrenocortical adenoma. Primary adrenocortical insufficiency without hypoaldosteronism has occasionally been observed, particularly in the familial form of the disease.

Selective hypoaldosteronism or analdosteronism occurs rarely. Theoretically, there are two possible enzyme deficiencies which can lead to congenital isolated hypoaldosteronism: these are 18-hydroxylase and 18-hydroxydehydrogenase deficiencies. The patients with isolated hypoaldosteronism and salt loss described by VISSER and COST (1964) were probably suffering from 18-hydroxylase deficiency due to a hereditary autosomal recessive defect.

Congenital hypoaldosteronism and salt loss were attributed to 18-hydroxydehydrogenase deficiency in a case described by ULICK (1964) because the secretion rate of 18-hydroxycorticosterone was found to be increased. An impairment of aldosterone biosynthesis at the level of 21-hydroxylation is at least partly responsible for the salt-losing syndrome observed in certain cases of the congenital adrenogenital syndrome (see p. 369f.). This appears to be a separate disease and also to be genotypically different from simple virilizing 21-hydroxylase deficiency, in which the aldosterone secretion rate is normal or elevated.

Several cases of acquired selective hypoaldosteronism have been described. The pathogenesis is uncertain. Particularly in the cases in which orthostatic hypotension was the predominant clinical feature, the decreased aldosterone secretion rate was probably due to impaired secretion of renin rather than a primary adrenocortical disorder (see Chap. XV) (SLATON, 1967). A decreased aldosterone secretion rate has been observed in cases of pretectal brain tumors. Long-term treatment with heparin, which inhibits aldosterone biosynthesis by an unknown mechanism, can lead to hypoaldosteronism with orthostatic hypotension.

h) Secondary Adrenocortical Insufficiency

α) Panhypopituitarism

Whereas primary insufficiency is due to damage of the adrenals, secondary adrenal insufficiency is caused by pituitary corticotropin deficiency. The adrenal insufficiency in panhypopituitarism is a prototype of this form (see p. 91ff.). As mentioned above, patients with secondary adrenal insufficiency differ from normals and Addisonian patients by a marked pallor of the skin; the disease is further characterized by a preferential failure of cortisol and androgens associated with intact aldosterone secretion.

β) Congenital Adrenal Hypoplasia

Congenital adrenal hypoplasia is regularly found in anencephaly and is associated with underdevelopment and malformation of the pituitary gland. Children with this condition usually die within the first few days of life. Only a few cases of congenital adrenal hypoplasia have been observed in which brain development was normal. The most frequent symptoms, appearing shortly after birth or within a few days or weeks, are anorexia, vomiting, insufficient weight gain, dehydration, cyanosis, and circulatory failure, all of which could as well be due to gastrointestinal or cardiac disorders. Addisonian pigmentation may appear within the first few months of life, which may possibly indicate a primary adrenocortical disorder. As in Addison's disease, serum sodium and glucose concentrations are decreased, whereas the potassium concentration is elevated. Steroid excretion at this age is so low in any case that a distinction between normal and decreased values is very difficult. Death usually occurs within days or months. When the disease is suspected,

oral substitution therapy with a daily dose of 12.5 to 25 mg of cortisone and 1 to 2 g of sodium chloride should be tried. A few cases of isolated glucocorticoid and androgen deficiency have been described (STEMPFEL, 1960). However, similar symptoms and electrolyte disorders in early infancy are more frequently caused by *congenital adrenocortical hyperplasia* (*adrenogenital syndrome with salt loss*, see p. 369 f.). The main characteristics of this disorder are malformation of the female genitalia and an elevated excretion of 17-ketosteroids. Secondary adrenal insufficiency without failure of the other glands regulated by the pituitary, and thus due to an isolated ACTH deficiency may occur spontaneously in rare instances (ODELL, 1960).

γ) Adrenal Insufficiency due to Cortisone Therapy

Adrenal insufficiency secondary to treatment with cortisone, its derivatives, or ACTH, or occurring after surgical removal of a cortisol-producing adrenocortical adenoma, is of great clinical importance. Adrenal insufficiency lasting several days is induced by treatment with cortisone for over a week in a dosage of over 75 mg per day, or with over 15 mg of prednisone given in three to four daily doses. The adrenals secrete a minimal amount of cortisol and are less responsive to ACTH. Although the responsiveness becomes normal within two to three days, the cortisol secretion rate does not exceed basal values since the pituitary reserve of ACTH is minimal. Maximal adrenocortical atrophy can be induced by a dose of 60 mg of cortisol within 15 to 20 weeks. Adrenocortical insufficiency may then persist for weeks and occasionally up to 6 months. As in latent Addison's disease, the patients' resistance is low, and death due to stress has been described. Although the extent and duration of secondary adrenal insufficiency are dependent on the duration and dosage of steroid therapy to some extent, individual factors appear to play a major role. Even the site of the disorder is not the same in all patients. In many patients, there is a temporary phase of hyporeactivity to ACTH and the increase in the plasma ACTH concentration precedes the increase of the plasma cortisol concentration for a few months (GRABER, 1965). But in other cases, deficient ACTH secretion is the predominant disorder (CARREON, 1960; JASANI, 1967). Several cases have also been observed in which no adrenocortical insufficiency was demonstrable after years of corticosteroid therapy in high doses.

A corticosteroid preparation for intravenous administration should always be to hand when patients treated with cortisone up to 6 months previously are subjected to surgery. Preventive corticosteroid therapy is not recommended as a general rule, however, since only 5 to 10% of patients treated with cortisone respond to surgical stress with an inadequate increase in cortisol secretion. The cortisone withdrawal syndrome can, but need not, lead to fatigue and anorexia, nausea and fainting. There is no adrenocortical atrophy after ACTH therapy, but the ACTH reserve of the pituitary is reduced. Serious incidents have been rarely observed. It is advisable to withdraw cortisone or prednisone gradually. In order to reestablish the diurnal variation of plasma cortisol, the night dose is withdrawn first, followed by the evening dose while administration of a single morning dose is continued. Intramuscular injection of long-acting ACTH preparations for 3 to 5 days after or during cortisone withdrawal restores adrenocortical responsiveness, but is of little benefit, since it inhibits pituitary ACTH secretion further.

It has recently been recommended that high pharmacological doses of cortisone be administered intermittently as a single morning dose on alternate days. Another recommendation is that synthetic glucocorticoids with a long half-life be given once daily in the morning. With these methods Corticotrophin secretion recovers after 12 and 36 hours respectively, and adrenal atrophy is thought to be avoided without

Table 7. Synopsis of symptoms and tests in various forms of adrenocortical insufficiency

		Pigmentation	Electrolyte disturbances	Hypoglycemia	Decreased urinary steroids	ACTH tests
Primary:	Addison's disease	+ + +	+	+ +	+ +	−
	Latent Addison's disease	+ +	−	−	+	−
Secondary:	Pituitary insufficiency	− (Pallor)	−	+ + +	+ + +	+ Repeated on 3 successive days i.v.
	Addisonism	−	−	−	+	+ + +

impairment of the therapeutic effect of the corticosteroid therapy.

i) "Addisonism", "Relative Adrenal Insufficiency" ("Benign Hypoadrenia")

A syndrome consisting of rather nonspecific symptoms vaguely reminiscent of ADDISON's disease, such as fatigue, hypotension, weight loss, and tendency to hypoglycemia, may occur from unknown causes or may accompany severe acute or chronic illnesses. In the past, such a condition was often assumed to be due to toxic damage of the adrenals and was refered to by such terms as "Addisonism" (THADDEA) or "benign hypoadrenia" (GOLDZIEHER). This assumption, however, is no longer acceptable, since the adrenals always respond normally to ACTH in these cases. On the other hand, low urinary steroids, which are often observed during severe acute and chronic illnesses as well as in convalescence and anorexia nervosa, must be due either to inadequate pituitary stimulation or to altered metabolism, because they increase normally in response to ACTH.

These conditions are clinically totally different from Addison's disease, latent Addison's disease or secondary insufficiency in panhypopituitarism or after cortisone therapy. They never lead to acute adrenal failure or to dangerous crises. Hormone therapy is contraindicated (see p. 329). The organism is always capable of satisfying increasing demands and adapting even to severe stress. With the exception of low urinary steroids, laboratory findings indicate normal adrenocortical function.

A diagnosis of adrenal insufficiency must never be based solely on noncharacteristic symptoms such as fatigue, hypotension, and weight loss. "Addisonism", as characterized by the symptoms of any severe illness, is not a pathologic entity and is of no clinical importance. Diagnoses of "Addisonism" and "benign hypoadrenia" should be avoided, because they may lead to unnecessary hormone treatment.

Pathologic anatomical findings of "Addisonism" have of course never been described. In one large series of autopsies, such findings as turbid swelling, cystic degeneration, and thrombotic changes of the adrenals were attributed to "benign hypoadrenia" (GOLDZIEHER), but adrenal insufficiency was not clinically substantiated. For similar reasons, the question of to what extent the status thymolymphaticus is due to functional disorders of the pituitary-adrenocortical system is also still unsolved, since the pathologic anatomical findings have never yet been correlated with the results of endocrine function tests in the same patients.

4. Differential Diagnosis of Adrenocortical Insufficiency

Diagnosis can be very easy in fully developed Addison's disease, but very difficult when the symptomatology is incomplete. The differentiation between sprue and Addison's disease can be particularly difficult. The clinical picture of these two conditions can be so similar that differentiation is only possible with the help of laboratory tests. Patients with sprue complain of fatigue, hypotension, weight loss, and diarrhea, which are also characteristic symptoms of Addison's disease. Moreover, objective signs, such as scanty pubic and axillary hair, particularly in women, and even pigmentation can be observed. Pigmentation of sprue, however, can usually be distinguished from Addisonian's pigmentation, because it is dirty gray in coloar, chloasma-like, and localized around the mouth and on the forehead without affecting the buccal mucosa and palmar lines. The diarrhea in sprue is characterized by voluminous, grey-yellow, offensive-smelling, mushy, fatty stools with a greasy gloss. Anemia is more severe in sprue than in Addison's disease and finally, sprue can be suspected when bone pains, indicating osteomalacia, and Chvostek's and Trousseau's signs, indicating tetany, are present. In sprue, the adrenals always respond normally to ACTH. Urinary steroids may be decreased, but the functional reserve of the adrenals is intact. No disorders of sodium and potassium balance or carbohydrate metabolism are demonstrable. A flat blood sugar curve obtained in the oral glucose tolerance test may be due to impaired absorption, as suggested by the normal results of an intravenous glucose tolerance test or an insulin tolerance test.

Chronic interstitial nephritis also has many clinical features in common with Addison's disease. This condition is mainly characterized by tubular damage and is referred to by such terms as renal acidosis, Lightwood-Albright syndrome, diabetes salinus renalis, or salt-losing nephritis, depending on the electrolyte disorder. In this disease, as in Addison's disease, a severe loss of sodium and also of chloride is possible, but it is not due to a deficiency of aldosterone, but to the damaged target organ, the tubules, which can no longer respond to the hormone.

Asthenia, hypotension, anorexia, and anemia are the predominant clinical features of diabetes salinus renalis. Tubular damage can also lead to potassium and calcium deficiency, and thus to pareses and renal osteomalacia. Surprisingly, diffuse and localized pigmentation of the skin, and even buccal mucosal pigmen-

tation of the Addisonian type, may be observed. Its pathogenesis is unexplained. Laboratory findings always permit a differential diagnosis: whereas serum urea nitrogen and nonprotein nitrogen can be elevated in both diseases, serum potassium is increased in Addison's disease and initially normal or decreased in salt-losing nephritis. The ACTH test is always decisive, because it is abnormal only in primary adrenocortical insufficiency. Hypo- and isosthenuria, severe metabolic acidosis, and delayed phenolphthalein excretion are indicative of chronic interstitial nephritis. A patient with renal disease is unable to concentrate his urine, whereas the dilution test is pathologic in adrenocortical insufficiency.

Differential diagnosis concerns the following features of Addison's disease: increased pigmentation is found in sprue and certain forms of nephritis as well as in chronic gastrointestinal disorders (melanosis of gastrointestinal polyposis, Peutz-Jeger syndrome Cronkhite-Canada syndrome), in pregnancy, and during treatment with hormonal inhibitors of ovulation. Persistent hyperthyroidism can also cause pigmentation, due to increased ACTH secretion induced by accelerated metabolism of cortisol. Cirrhosis of the liver can lead to pigmentation similar to that in Addison's disease. Pigmentation due to exogenous causes such as chronic ultraviolet radiation, physical irritation of the skin (cutis vagantium) and vitamin deficiency (pellagra) may be reminiscent of Addison's disease. In rare cases, a metastasizing melanoma may lead to diffuse cutaneous pigmentation. Iatrogenic pigmentation of the skin can result from chronic administration of hydantoin preparations, from alkylating cytostatic therapy of Hodgkin's disease, and also from administration of arsenic, bismuth, gold, and silver. The blue lines induced by chronic lead intoxication have to be differentiated from buccal mucosal Addisonian pigmentation. Biopsies are indicated in doubtful cases. The skin pigmentation of hemochromatosis can usually be distinguished from Addisonian pigmentation by its grayish violet tinge. However, differentiation can be difficult, since melanin is the major pigment present in hemochromatosis also, and iron deposits are not always histologically demonstrable. Hemochromatosis can be differentiated from Addison's disease by the enlarged firm liver and by the diabetes-like disorder of carbohydrate metabolism. Vitiliginous pigmentation can occur in neurofibromatosis and in polyostotic dysplasia with pubertas praecox (Albright's syndrome, see Chap. XIX).

Asthenia is a symptom associated with most illnesses and by itself does not justify suspicion of Addison's disease. The asthenia of neurasthenia and of anorexia nervosa may sometimes be suggestive of Addison's disease. However, neurasthenic symptoms do not have the characteristics of organic fatigue. The ACTH test permits a clear differentiation in ambiguous cases. All chronic infections, particularly tuberculosis and brucellosis, can lead to the type of organic fatigue usually found in Addison's disease.

It is sometimes difficult to differentiate true Addison's disease from the secondary adrenal insufficiency of Simmond's disease, although Addisonian patients are hyperpigmented, whereas patients with hypopituitarism are pale. Secondary adrenal insufficiency can be recognized by hypothyroidism, complete absence of the axillary and pubic hair, absence of electrolyte disorders, and a positive response to the intravenous ACTH test repeated on 3 days.

Adynamia, pareses, and paralysis of myasthenia gravis are immediately alleviated by treatment with prostigmine and tensilone. Myopathy in the apathic type of thyrotoxicosis may resemble Addison's disease, especially since the patients are hyperpigmented. Fatigue and atony combined with increased irritability are common to hyperparathyroidism and Addison's disease.

Hypoglycemia with blood sugar values below 60 mg% can also be due to hypopituitarism and to hyperinsulinism in the adult (see Chap. XIII). Adenomas and hyperplasia of the islet cells, however, lead to overweight and a healthy appearance. In vegetative lability, reactive hypoglycemia never reaches values below 50 mg%. Finally, in childhood, glycogen storage disease and the so-called spontaneous hypoglycemia have to be considered in the differential diagnosis.

Hypotension is very common and by itself does not suggest Addison's disease. It can be due to constitutional factors and is not necessarily a sign of other diseases. See p. 426 for a discussion of orthostatic hypotension due to adrenal medullary insufficiency.

5. Therapy of Adrenal Insufficiency

a) Diet

Dietary therapy with a low-potassium diet and a daily salt intake of 10 to 15 g was important before pure hormones were available. Today, Addisonian patients do not require any special diet.

b) Organ Transplantation

Organ transplantation is completely outdated, at least for the present. Animal adrenal glands

contain only a fraction of the necessary therapeutic dose of cortical hormones and are soon rejected or resorbed by the organism. The few cases of successful homotransplantation are of only theoretic interest. Only when the immunological problems associated with transplantation surgery have been successfully solved will interest in the treatment of primary adrenocortical insufficiency by organ transplants be renewed.

c) Adrenocortical Hormone Preparations

Adrenal tissue extracts are no longer in use.

Deoxycorticosterone has a purely mineralocorticoid effect. Overdosage causes edema, hypertension, and hypokalemia. Deoxycorticosterone acetate in sesame oil is given intramuscularly. A microcrystalline suspension of trimethylacetate esters of deoxycorticosterone has a prolonged activity over 4 weeks. After a single injection of 30 mg, approximately 1 mg per day is absorbed.

Cortisone has a pronounced glucocorticoid and a weak mineralocorticoid activity. Cortisone acetate tablets are administered orally. In contrast to deoxycorticosterone, oral cortisone is completely resorbed, enters the blood stream quickly, and persists for 8 to 12 hours in a sufficient concentration. Oral administration is therefore the method of choice.

Cortisol, like cortisone, has a potent glucocorticoid and a weak mineralocorticoid activity. It is 25 percent more effective than cortisone.

Cortisol acetate tablets are resorbed more quickly than cortisone acetate. In long-term maintenance therapy it has therefore no advantages over cortisone, since it must be taken every 6 hours instead of every 12 hours. It can be used in preference to cortisone when resorption is insufficient or when fast action is required.

Cortisol hemisuccinate can be administered intramuscularly and intravenously even in high concentrations. Intramuscular injection results in an adequate plasma concentration for several hours. During operations and in acute emergencies, it must always be given in an intravenous infusion.

9α-Fluorocortisol is available as the acetate in tablets. It has a strong glucocorticoid and a very strong mineralocorticoid activity. A daily oral dose of 2 mg is sufficient for complete adrenocortical substitution therapy, but leads to sodium retention. A daily dose of 0.25 mg maintains electrolyte balance. Full substitution therapy is provided by 0.1 mg of fluorocortisol combined with 25 mg of cortisone acetate.

d) Substitution Therapy in Addison's Disease (Primary Adrenal Insufficiency)

Modern substitution therapy is simple and rewarding. Weak, bed-ridden patients regain their vigor and can lead a completely normal life. They recover the capacity to perform even hard physical and intellectual work and to take part in sport.

The standard therapy is 25 to 37.5 mg of cortisone acetate orally and 0.1 mg of fluorocortisol orally. Cortisone tablets are taken in two daily divided doses, one at breakfast and one at 4 p.m. to simulate the physiological diurnal variation in plasma concentrations. We usually give 1 tablet of 25 mg of cortisone and 1 tablet of 0.1 mg of fluorocortisol in the morning, and one half tablet of cortisone (12.5 mg) in the afternoon. Often 25 mg of cortisone is sufficient, in rare instances even 12.5 mg. In general, the smallest possible dose which maintains well-being and maximal efficiency should be given. A daily dose of 0.05 to 0.2 mg of fluorocortisol is generally needed. Overdosage leads to edema, and underdosage to weight loss and hypotension. In heart failure, it is sometimes advisable to withdraw fluorocortisol, but to permit unrestricted salt intake.

Instead of fluorocortisol, an intramuscular injection of 25 mg of trimethylcortexone in crystalline suspension can be given every 3 to 4 weeks. Finally, a daily supplement of 5 to 10 g of salt can replace the mineralocorticoids. The natural mineralocorticoid d-aldosterone would have to be given intramuscularly as an acetate in an oily solution in a daily dose of 75 to 150 μg. The maintenance dose of aldosterone would be 20 to 40 times higher for oral administration and 2 to 6 times higher for sublingual administration. Aldosterone is now only rarely used for maintenance therapy.

The use of prednisone or other synthetic steroids instead of cortisone is pointless, since they have no sodium-retaining activity and thus necessitate higher doses of mineralocorticoids at the same time.

Whenever parenteral hormone administration is indicated, cortisol hemisuccinate should be given i.m. twice daily and 2.5 mg of deoxycorticosterone daily by i.m. administration. When cortisol hemisuccinate is not available, 5 mg of prednisolone hemisuccinate or phthalate can be given twice daily as well as 5 to 10 mg of deoxycorticosterone intramuscularly.

It is not advisable to administer cortisone acetate by i.m. injection as a suspension, because it is absorbed too slowly. Tablet implantation has also been discontinued.

In the woman, adrenocortical failure leads to a complete lack of androgens. They do not usually need to be replaced. It is not known whether they have any influence on the psyche. Loss of libido justifies a trial therapy with fluoxymesterone, 2.5 to 5 mg orally.

e) Treatment of Complications

Any additional stress induced by physical exertion, trauma, or acute illness increases the requirement for cortisone. Addisonian patients must be aware that they are exposed to serious danger by every injury, infection, and operation, and they must learn to meet this danger by increasing the cortisone dose. They should always carry a supply of cortisone tablets on them and if possible, cortisol for i.m. injection also. A dose of 75 to 150 mg of cortisone must be taken orally at once in febrile illnesses. If vomiting occurs, a doctor should be consulted and 50 to 100 mg of cortisol hemisuccinate should be given i.m. twice daily. Deoxycorticosterone acetate should be given in a dose of 5 to 10 mg i.m. in oily solution when signs of dehydration are observed. Immediate hospitalization is indicated in every severe illness.

Cortisone is not contraindicated in active or inactive tuberculosis treated with tuberculostatic drugs. However, the patient should be carefully supervised. The unequivocally favorable effect on the general condition outweighs possible impairment of the induration process. However, when cortisone has a clearly negative effect, the patients must be maintained on deoxycorticosterone alone. Under this therapy, they are considerably less resistant to stress, and particular caution is indicated during anesthesia and when morphine preparations are used. In view of the disturbed carbohydrate metabolism, frequent small meals are indicated. A snack containing 50 g of carbohydrate and 25 g of protein should be taken before retiring.

f) Substitution Therapy during Surgery and during Adrenalectomy

Today, even extensive surgery is possible in Addisonian patients under the cover of an intravenous cortisol infusion without a higher risk than in normals. Even total adrenalectomy is not hazardous when performed under adequate substitution therapy. Adrenalectomy in Cushing's syndrome requires a higher dose of cortisol and a slower reduction to the maintenance dose, since the organism has been overloaded with cortisol.

The treatment is outlined in Table 8.

Table 8. Adrenocortical substitution during surgery

1. *Substitution therapy for minor operations (tooth extraction)*
   1 hour before, cortisone acetate, 75 to 100 mg p.o.

2. *Substitution therapy during adrenalectomy and during surgery in patients with adrenal insufficiency*
   Starting from the beginning of anesthesia, intravenous infusion of 1 liter of 2:1 physiological glucose solution/physiological NaCl solution with cortisol hemisuccinate, 100 mg, over 8 hours. During the following 16 hours, again 1 liter of the same 2:1 infusion solution with cortisol hemisuccinate, 100 mg. In hypotension (less than 100 mm Hg systolic) 2nd infusion with L-noradrenaline, 10 mg, or angiotensin II, 10 mg, in 500 ml physiological sodium chloride solution at a rate of 5 to 40 drops per minute, i.e. 0.3 to 2.4 mg of L-noradrenaline and angiotensin respectively per hour. Infusion must never be interrupted.
   1st postoperative day:
   2 liters of 2:1 solution with cortisol hemisuccinate, 50 mg per liter, i.v.
   2nd postoperative day:
   If possible, switch to oral cortisone acetate, 4 × 25 mg.
   3rd postoperative day:
   cortisone p.o., 3 × 25 mg.
   Thereafter, gradual reduction over 3 to 6 days according to clinical criteria to the maintenance substitution dose of 25 to 37.5 mg of cortisone acetate p.o. and 0.1 mg of fluorocortisol p.o.

3. *Substitution therapy during adrenalectomy for Cushing's syndrome*
   Starting from the beginning of anesthesia, an intravenous infusion of 1 liter of 2:1 physiological glucose/physiological saline solution with cortisol hemisuccinate, 200 mg, over 8 hours. In the following 16 hours, 1 liter of the same 2:1 infusion solution with 200 mg of cortisol hemisuccinate.
   In hypotension (less than 100 mm Hg systolic) 2nd infusion with L-noradrenaline, 10 mg, or angiotensin II, 10 mg, in 500 ml of physiological saline solution at a rate of 5 to 40 drops per minute, i.e. 0.3 to 2.4 mg of L-noradrenaline and angiotensin II respectively per hour. The infusion must never be interrupted.
   1st postoperative day:
   2 liters of 2:1 solution with 100 mg of cortisol per liter, i.v.
   2nd postoperative day:
   2 liters of 2:1 solution with cortisol, 100 mg per liter, i.v.
   3rd postoperative day:
   If possible, switch to oral cortisone acetate in a dose of 4 × 50 mg.
   4th postoperative day:
   3 × 50 mg of cortisone, p.o.
   Thereafter, gradual reduction over 2 to 3 weeks according to clinical criteria to a maintenance substitution dose of 25 to 35 mg of cortisone acetate p.o. and 0.1 mg of fluorocortisol p.o.

g) Treatment of Acute Adrenal Insufficiency

Addisonian Crisis. When chronic adrenal insufficiency exacerbates into acute crisis under the influence of infection, trauma or surgery, the patient's life is in serious danger and immediate intensive treatment is necessary. Every delay and every stressful diagnostic procedure can cause death. When the diagnosis is uncertain

and the patient is in a relatively good condition, a blood sample may be taken for the determination of potassium, sodium, chlorides, urea, cortisol, and blood sugar. Afterwards, the following therapeutic procedure should be followed:

Addisonian crisis in a patient treated or overtreated with deoxycorticosterone alone is characterized by symptoms of hypoglycemia together with signs of water retention. There may be edema, high blood pressure, coma, epileptic seizures, and pulmonary edema. Sodium chloride and fluid administration are contraindicated in these cases. Intravenous administration of cortisol in 10% glucose solution and plasma infusions are indicated. If hypokalemia has been demonstrated, KCl, 6 g, is given in enteric-coated tablets or an intravenous infusion of 2 g of KCl in 1000 ml of 5% glucose solution is given within one hour.

Table 9. Therapy of Addisonian crisis (for children reduce doses by 0–50%)

1. Intravenous infusion over 3 hours of 100 mg of cortisol hemisuccinate in 500 ml of physiological saline solution to which 50 ml of a 40% glucose solution can be added. If no water-soluble cortisol preparation is available, prednisolone hemisuccinate or prednisolone phthalate, 25 mg. If intravenous infusion is impossible, cortisol hemisuccinate, 100 mg or prednisolone hemisuccinate, 25 mg, i.m.
2. In hypotension (systolic blood pressure below 100 mm Hg), infusion of 10 mg of noradrenaline or angiotensin II in 500 ml of physiological saline solution at a rate of 5 to 40 drops per minute, i.e. 0.3 to 2.4 mg per hour of L-noradrenaline or angiotensin. It may be advisable to give 500 ml of plasma or blood after rehydration.
3. Continued intravenous infusion of cortisol, 10 mg per hour, until patient is able to drink and to take cortisone tablets orally (4 × 50 mg per day).
4. Gradual reduction of cortisone to maintenance dosage of 25 to 37.5 mg of cortisone p.o. and 0.1 mg of fluorocortisol p.o. within 3 to 6 days.
5. Antibiotics may be necessary.

h) Therapy of Latent Addison's Disease

In these patients, the reduced endogenous production of adrenocortical hormones is adequate to meet the requirements caused by a normal life style. Blood pressure and weight, but mainly the general condition and efficiency indicate whether and in what dosage long-acting deoxycorticosterone, fluorocortisol or cortisol should be given. Even when hormones are not usually needed, the patients must always carry cortisone on them, so that they can take 100 to 150 mg of cortisone at once in case of infection or trauma. If acute insufficiency develops, it must be treated in the same way as the Addisonian crisis.

i) Therapy of Secondary Adrenal Insufficiency in Panhypopituitarism and after Cortisone Treatment

Adrenal atrophy due to ACTH deficiency can usually be reversed by intensive ACTH stimulation even after years. However, maintenance therapy with ACTH has not been very satisfactory, because ACTH always has to be given by injection, whereas cortisone can be taken orally.

In secondary adrenal insufficiency, the secretion of mineralocorticoids is normal under basal conditions. Cortisone alone is sufficient for full substitution therapy. The basal secretion of cortisol may be adequate for a *vita minima*. Every additional stress can precipitate a crisis in these patients. In addition to small doses of cortisone acetate (12.5 to 25 mg p.o.) in panhypopituitarism, sodium-l-thyroxine must be given for the thyroid deficiency and testosterone for male gonadal insufficiency (see p. 105).

See p. 326 for a discussion of secondary adrenal insufficiency after long-term treatment with high doses of cortisone.

When a sudden stress precipitates these patients into acute adrenal insufficiency, cortisol must be given intravenously at once, as in Addisonian crisis, although it promotes further atrophy. ACTH, even when given intravenously, cannot stimulate the atrophic adrenals fast enough to be of use in an emergency situation, and thus cannot replace cortisone. Children receive between half and the full adult dose.

6. Prognosis of Adrenal Insufficiency

The prognosis of Addison's disease has changed completely with the development of therapy. Before 1930, when no specific treatment was available, 63% of Addisonian patients died within $1^1/_2$ years. Between 1930 and 1937, with treatment with salt and adrenal extracts, only 43% died within $1^1/_2$ years. Since deoxycorticosterone has been added to the therapy, only 14% have died within $1^1/_2$ years. The introduction of cortisone has lowered the mortality further. Prognosis is further influenced by additional diseases, such as urogenital tuberculosis. Simultaneous pulmonary tuberculosis is rare. Addison's disease due to adrenal atrophy is thought to have a more favorable prognosis than the tuberculous form of the illness. A slow onset of the symptoms also indicates a better prognosis. Patients who only show an increase in pigmentation and hypo-

tension over a long period of time and have no gastrointestinal or hypoglycemic symptoms may live without hormone therapy for many years. A tendency to hypoglycemia is an ominous sign which calls for immediate treatment. The prognosis, however, is mainly a function of the therapy and the care with which it is carried out. Patients have to be given as much information as possible about their illness. They must be taught to recognize their symptoms and to act accordingly. After the establishment of optimal maintenance therapy we make a point of seeing and examining the patient at first every three months and later once a year.

F. Hyperfunction of the Adrenal Cortex

1. Classification

As shown on p. 288 and in Table 1, the human adrenal cortex produces mainly cortisol, corticosterone, and dehydroepiandrosterone sulfate. Aldosterone, which is produced in small amounts, is of great physiological importance, whereas the other less active or inactive precursors or by-products of adrenocortical hormones which are found in the blood have no clinical significance under normal conditions. Secretion of small amounts of testosterone is proven, and estrogens are probably also secreted.

Thus there are three distinct clinical syndromes of hyperfunction:

1. Excess of mineralocorticoids (primary aldosteronism, Conn's syndrome);
2. Excess of cortisol (Cushing's syndrome);
3. Excess of androgens (adrenogenital syndrome).

A combination of Cushing's syndrome and adrenogenital syndrome can be due to an adrenal adenoma. Combinations of all three syndromes, particularly characterized by massive secretion of precursors, can be caused by adrenal carcinoma. Hirsutism and acne are signs of pure Cushing's syndrome, whereas virilism indicates an associated adrenogenital syndrome.

2. Mineralocorticoid Excess

a) Primary Aldosteronism (Conn's Syndrome)

α) Definition, Incidence

The term primary aldosteronism designates autonomous overproduction of aldosterone by the adrenals. The disease is always caused by an aldosterone-producing tumor of the adrenal cortex* ("aldosteroma", "aldosteronoma"). The classic Conn's syndrome consisted of the symptoms of hypokalemia, such as muscular weakness or periodic pareses and polyuria, and of benign hypertension. However, CONN no longer considers hypokalemia a main feature of the disease. In many patients, hypertension precedes hypokalemia by several years, and CONN has been able to diagnose primary aldosteronism in a few normokalemic hypertensive patients from laboratory findings showing an increased aldosterone secretion rate and a decreased plasma renin activity. However, only very few cases of normokalemic primary aldosteronism have been diagnosed so far.

The incidence of the disease is also still very controversial. In the decade following Conn's first description of the illness (1955), a few hundred cases were described in the literature. Until 1965, primary aldosteronism was considered to be a rare cause of hypertension. In the United States, where about 17 million inhabitants suffer from hypertension, less than 1000 cases of primary aldosteronism had been diagnosed. CONN (1965/1966), however, suggested that actually at least 20% of all patients with "essential" hypertension suffered from undiagnosed primary aldosteronism. This hypothesis was based on the incidence of a raised aldosterone excretion rate in patients with essential hypertension (GARST, 1960) and on the incidence of adrenocortical adenomas found at autopsy of patients with essential hypertension. Although it is quite possible that the disease will be more frequently diagnosed in future due to improved laboratory methods and mass screening of hypertensive patients, evidence presented by a number of other investigators is not compatible with Conn's hypothesis. According to clinical studies carried out with double isotope assays, aldosterone secretion rate is normal in the majority of patients with benign hypertension. LARAGH (1966) did not find a single case of increased aldosterone secretion among 73 patients with benign essential hypertension. Aldosterone excretion rate was normal in 43 patients with benign hypertension studied by KAPLAN (1967). In a prospective study carried out in 90 unselected cases of "essential" hypertension, primary aldosteronism could be excluded by a normal aldosterone secretion rate, a normal plasma renin activity, or both in 87 patients. By contrast,

* Some authors use the term "primary aldosteronism" also for cases of aldosterone excess of unknown etiology associated with bilateral adrenocortical hyperplasia and a decreased plasma renin concentration (p. 339). According to some authors, this disease is as frequent as tumorous primary aldosteronism (GEORGE, 1970).

primary aldosteronism was found in 4 out of 10 patients with hypertension and hypokalemia (FISHMAN, 1968). Moreover, an adrenocortical adenoma found at operation or autopsy of a hypertensive patient does not prove that the hypertension was of adrenal origin. The steroid content of adenomas of patients with essential hypertension was normal, whereas adenomas of patients with primary aldosteronism contained 10 to 100 times more aldosterone and 10 times more corticosterone per unit weight than normal human adrenocortical tissue. Until proved otherwise, primary aldosteronism must therefore still be considered a rare cause of hypertension.

The female sex is affected $2^1/_2$ times more frequently. Two thirds of cases are diagnosed during the 4th and 5th decade of life. The disease can, however, occur at any age. Although juvenile "congenital aldosteronism" with bilateral adrenal hyperplasia is a form of secondary aldosteronism (see p. 339), several cases of primary aldosteronism due to adrenal adenomas during childhood have been described.

β) Pathologic Anatomy

The adenomas occur 2 to 3 times more frequently on the left side than on the right. In 90% of the cases, they are solitary, in 10% they are multiple, and in only 2% are they bilateral. They are usually very small and can therefore not be diagnosed radiologically. The weight of 63% of the adenomas is less than 6 g, and the greatest diameter is less than 3 cm in 86%.

They are composed of cells reminiscent of the zona fasciculata. The adenoma itself and the adjacent cortical tissue show no specific alterations indicative of mineralocorticoid excess. Atrophy of the remaining cortex has, however, been described.

Considerable amounts of aldosterone can be isolated from the tumors, whereas the aldosterone content of the adjacent adrenal tissue is strikingly low. In most cases, however, corticosterone and not aldosterone is found to be the prevalent steroid in tumors causing primary aldosteronism. Considerable amounts of cortisol are also often found which could explain the contralateral atrophy observed in some cases, necessitating temporary substitution therapy.

The kidneys usually show typical signs of hypokalemic nephropathy. In addition, arteriosclerosis resulting from hypertension is often found. Signs of chronic pyelonephritis are not uncommonly observed.

γ) Clinical Features

The leading symptoms are caused by *hypertension* and *hypokalemia*. Headache due to hypertension is rarely absent. Potassium is lost mainly in the urine, but also in the colon (SHIELDS, 1968). Hypokalemia leads to muscular weakness, which may be constant or, frequently, progressing to periodic pareses and paralyses, and also to polyuria, especially during the night, accompanied by isosthenuria and polydipsia, which are not always very pronounced (see Chap. III, p. 56).

Tetany is often, but not always present and, like hypocalcemic tetany in the latent stage can be recognized by positive Chvostek's and Trousseau's signs. It is due either to metabolic alkalosis or to magnesium deficiency. There is usually no edema. Only a few cases of classic Conn's syndrome with edema have been described and a special reason for the edema was found in most of them.

Paresthesia is not uncommon and is probably due to the alkalosis.

Table 10. Frequency of clinical manifestations of primary aldosteronism. (From KOCZOREK, 1964)

a) *Symptoms*	
Polyuria, nycturia	73%
Muscular weakness	71%
Headache	53%
Polydipsia	48%
Intermittent paralysis	25%
Parasthesia	24%
Tetanic seizures	21%
No symptoms	5%
b) *Clinical findings*	
Benign hypertension	100%
Retinopathy I to III	53%
Increased heart size	42%
Trousseau	17%
Chvostek	8%
Atonic pareses	4%

The hypertension is usually benign, although diastolic blood pressure may vary between 90 and 160 mm Hg. The heart is enlarged in only half the cases. Hypertension is not progressive, does not lead to arteriolar necrosis, and produces retinopathy of grade I to III. Papilledema is not found. The symptomatology may, however, be incomplete and there are known cases of surgically verified primary aldosteronism where there were no symptoms at all; the hypertension was discovered at a routine examination and hypokalemia was then detected.

δ) Laboratory Findings

1. Electrolyte Balance. In most cases the main laboratory finding is hypokalemia, which generally varies between 1.4 and 3.2 mEq/l. Normokalemic primary aldosteronism is rare. In most normolkalemic cases, hypokalemia can be provoked by oral sodium loading (200 mEq of Na^+ over several days). Potassium balance is negative and urinary potassium excretion is inappropriately high in relation to the low serum potassium level. The urinary potassium loss is substantiated by the fact that daily potassium excretion is not reduced to 20 mEq or less when a potassium-deficient diet (20 mEq daily) is given or when the serum potassium is below 3 mEq/l. Potassium excretion does not stop completely in healthy subjects, but it is considerably reduced. Characteristic changes in potassium balance can be induced by increase or reduction of sodium intake. When potassium intake is kept constant (100 mEq per day) and sodium intake is increased to 200 mEq per day, there is a marked rise in urinary potassium excretion and a further fall in serum potassium after a few days. This is due to a stimulated sodium-potassium exchange in the distal renal tubules under the influence of an elevated plasma aldosterone. Conversely, potassium excretion decreases and serum potassium rises when dietary sodium intake is restricted and no sodium is available for exchange with potassium in the distal tubules. In time, the negative potassium balance leads to a considerable deficit in total body potassium, and exchangeable body potassium as estimated with ^{42}K may fall to 70% of normal. A very high potassium intake (200 mEq per day) may reduce the potassium deficit to some extent, but does not usually normalize the hypokalemia.

A decrease in potassium excretion and a rise in serum potassium in response to spironolactone are characteristic of primary aldosteronism and help to differentiate this condition from tubular nephropathy with potassium loss.

Hypernatremia is not as common and not as pronounced as hypokalemia. Serum sodium varies between 137 and 160 mEq/l and overlaps with normal values. Exchangeable body sodium, as measured by ^{24}Na, is elevated by an average of 15%. However, interpretation of this parameter is difficult because of the sodium stored in the bones. The extracellular fluid volume is increased and the plasma volume shows an average increase of 15%. There may be some overlap with normal values. A definite decrease in serum sodium or in plasma volume indicates secondary rather than primary aldosteronism.

In primary aldosteronism, the sodium-potassium ratio is often more strikingly reduced in the sweat and saliva than in the urine. However, the sodium content of these two body fluids varies with their secretion rate, and standardization is difficult. The estimation of the sodium-potassium ratio in the sweat related to the secretion rate may be used as a simple diagnostic test for primary aldosteronism in mass screening of hypertensives (GRANDCHAMP, 1967).

Hypomagnesemia of uncertain origin is found in many cases and may increase the predisposition to tetany. The hypokalemia is accompanied by metabolic alkalosis with plasma bicarbonate levels between 24 and 54 mEq/l (average 35) and an increased plasma pH. Although aldosterone promotes the excretion of H-ions, the urine is usually alkaline with a pH between 7 and 7.5.

Polyuria, polydipsia, nycturia, and hyposthenuria are caused by the hypokalemia. The polyuria is not reduced by vasopressin and the urinary osmolality is always lower than the plasma osmolality.

2. Aldosterone. Proof of a pathologically elevated and relatively autonomous aldosterone secretion rate is one of the most important criteria for the diagnosis of primary aldosteronism. In most cases, estimation of urinary aldosterone-18-glucuronide hydrolyzable at pH 1, is sufficient. However, when aldosterone metabolism by the liver is impaired (liver diseases, pregnancy), the aldosterone secretion rate has to be determined. In most cases, the aldosterone secretion rate is moderately increased, to approximately twice the normal value. Massive increases in aldosterone secretion rate are more frequently found in secondary (malignant hypertension) than in primary aldosteronism. Electrolyte status must always be taken into consideration when interpreting aldosterone secretion or excretion rates. An elevated aldosterone excretion rate is pathologic only when the daily sodium intake exceeds 100 mEq (6 g of sodium chloride).

Aldosterone secretion rate in primary aldosteronism is characteristically not or only slightly inhibited by measures leading to increased plasma volume:

daily intake of 200 mEq of sodium (12 g of sodium chloride, possibly in the form of gelatine capsules) for 5 days;

infusion of 2 liters of physiologic saline solution given over 4 hours on each of two consecutive days;

parenteral administration of deoxycorticosterone acetate, 20 mg daily for 3 consecutive days, with normal salt intake.

However, it must be borne in mind that in edematous patients these measures only increase the extracellular fluid volume and have no effect on circulating plasma volume. In this type of secondary aldosteronism, aldosterone secretion is not suppressed, and autonomy may be simulated.

3. Renin, Angiotensin. Determinations of renin activity, renin concentration, or angiotensin II concentration in the plasma are the most important laboratory methods for differentiating primary from secondary aldosteronism, since the increased aldosterone secretion rate is directly attributable to an elevated renin-angiotensin level in almost all known forms of secondary aldosteronism. By contrast, in primary aldosteronism, renin activity, renin concentration, and angiotensin II concentration in the plasma are reduced or in the lower normal range and do not increase in response to sodium deficiency or orthostasis. When a direct estimation of plasma renin is not possible, an angiotensin infusion test may give indirect information about the activity of the patient's renin-angiotensin system. Patients with renovascular hypertension respond only to high doses of angiotensin II by an increase in blood pressure, whereas patients with primary aldosteronism are extremely sensitive and respond even to very low doses. The test, however, is not without risk in hypertensive patients.

4. Carbohydrate Metabolism. Whereas fasting blood sugar levels are usually normal, pathologically reduced glucose tolerance is found in 50% of patients with primary aldosteronism. The glucocorticoid activity of corticosterone, which is often secreted in excess, may contribute to this. It is, however, primarily due to the potassium deficiency. Hypokalemia impairs the formation of glycogen and retards insulin secretion.

ε) X-Ray Examinations

Retroperitoneal pneumoradiography is generally unsatisfactory for the preoperative lateralization of an aldosterone-producing adrenocortical adenoma, because small adenomas are not usually revealed by this technique. Better results can be obtained by selective adrenal phlebography (CONN, 1969). An unequivocal lateralization can be achieved by the determination of aldosterone in adrenal venous plasma from both sides, obtained by vena cava catheterization (MELBY, 1967). However, only very skillful and experienced radiologists are capable of catheterizing both adrenal veins. If preoperative lateralization is impossible or doubtful, the left adrenal should be operated upon first, because most aldosterone-producing adenomas are found on the left side (CONN, 1969).

Suprarenal aortography only occasionally reveals an adrenal tumor, but does disclose renal arterial stenosis.

Recently visualisation and localisation of the adenoma has been achieved by scintillation scanning after administration of ^{131}I-19-iodocholesterol (CONN, 1971).

ζ) Differential Diagnosis

Differential diagnosis of primary aldosteronism must eliminate other causes of first hypokalemia (Table 11) and secondly hypertension.

Table 11. Differential diagnosis of primary aldosteronism

	Blood pressure	Blood		Urine			Aldosterone	17-Hydroxy-corticoids
		K	HCO_3	Renin	K	Volume		
Primary aldosteronism	+		+	−	+	+	+	=
Cushing's syndrome	(+)	−	+		+	+	=	+
Malignant hypertension	+ + +	−	+	+	+	+	+ +	=
Renal hypertension	+ +	−	+	+	+	+	+ +	=
Tubular nephropathy	=	−	−	=	+	+	=	=
Chronic diarrhea Abuse of laxatives	−	−	+		−	−	−	=
Anorexia mentalis	−	−	+		+	−		−
Familial potassium-losing Nephropathy (LIDDLE)	+	−	+	−	+	+	−	=
Juxtaglomerular hyperplasia (BARTTER)	−	−	+	+	+	+	+	=
17-Hydroxylase deficiency	+	−	+	−	+	+	−	−
Iatrogenic:								
Salidiuretics		−	+	+	+	+	+	=
Cortexone	+	−	+	−	+	+	−	=
Liquorice juice	+	−	+	−	+	+	−	=

It must first be determined whether potassium is lost in the urine, in the stools, or by displacement into the intracellular space. As a rule, in primary aldosteronism more than 20 mEq of potassium are excreted in 24 hours, even when the diet is potassium-deficient or the serum potassium is below 3 mEq/l, unless the potassium reserves are completely exhausted after years of illness. Hypokalemia without hyperkaluria is typical of chronic diarrhea, or is suggestive of concealed abuse of laxatives. Much work can be saved by the demonstration of phenolphthalein in the feces or urine. Many laxatives contain phenolphthalein, and feces or urine turn red after alkalization. If salidiuretics are used in addition to laxatives because of the development of edema, the diagnosis becomes difficult, since hyperkaluria as well as an elevated aldosterone secretion rate may then develop. However, urinary potassium excretion decreases when salidiuretics are withdrawn for a few days. Hypokalemia with hyperkaluria and concurrent obstipation are found in anorexia mentalis (ROSSIER, 1955).

When hyperkaluria has been established, a tubular renal disorder has to be excluded. In the absence of a primary renal disease, hypokalemia is always associated with metabolic *alkalosis*, whereas metabolic acidosis is found in most tubular nephropathies, because they often cause impaired hydrogen ion excretion. On the other hand, hypokalemia of long standing can lead to impaired tubular epithelial function with the development of hyposthenuria. Renal disorders which may be associated with hypokalemia are renal acidosis, chronic interstitial nephritis, occasionally chronic pyelonephritis, and congenital tubular diseases, such as Fanconi's syndrome. Whereas these diseases are associated with normal or low blood pressure and metabolic acidosis, the syndrome of familial potassium-losing nephropathy described by LIDDLE (1964) leads to alkalosis and hypertension. The disease seems to be due to a generalized abnormality in membrane sodium transport (GARDNER, 1971).

Hyperkaluria together with metabolic alkalosis primarily suggests iatrogenic hypokalemia. Hypokalemia due to salidiuretic therapy in hypertension is often mistaken for primary aldosteronism. Salidiuretics promote sodium excretion, and thus lead to an increase in aldosterone secretion and excretion rate. *Liquorice juice* has a mineralocorticoid-like activity (see p. 303) and excessive intake (it is a component of certain soft drinks) leads to hypokalemia, hyperkaluria, alkalosis, and hypertension, but to a *decreased* aldosterone secretion rate. Similarly, a decreased aldosterone secretion rate is induced by chronic deoxycorticosterone acetate administration and is also found in Liddle's familial potassium-losing nephropathy.

Finally, when hypokalemia, alkalosis and hyperkaluria are associated with an increased secretion and excretion rate of aldosterone, primary aldosteronism has to be differentiated from secondary aldosteronism. Secondary aldosteronism due to idiopathic edema, nephrosis, cirrhosis of the liver with ascites, and cardiac failure is usually quite easy to differentiate from Conn's syndrome, because blood pressure is normal and severe hypokalemia is rare. It is more difficult to differentiate primary aldosteronism from malignant essential hypertension or renal hypertension due to unilateral stenosis of the renal arteries, which promote aldosterone secretion through the renin-angiotensin system and occasionally lead to adrenocortical hyperplasia. Extremely malignant, progressive hypertension with retinopathy grade IV and papilledema, is not likely to be due to primary aldosteronism, but has been observed in confirmed cases. Whereas hypernatremia is usually found in primary aldosteronism, serum sodium is normal or even decreased in malignant hypertension with secondary aldosteronism. In every case, intravenous pyelography, aortography, and possibly also HOWARD's test to assess the differential sodium excretion of both kidneys must be carried out to exclude renovascular hypertension. A correct preoperative diagnosis is the basis of successful surgery. When plasma renin or angiotensin levels can be determined, high values distinguish renal and malignant hypertension from primary aldosteronism with low renin and angiotensin levels. The angiotensin infusion test, which, however, is not completely harmless in hypertensive patients, can be used to differentiate essential hypertension and primary aldosteronism with a marked response from renal hypertension with a decreased response. In many patients with essential hypertension plasma renin activity is subnormal (FISHMAN, 1968).

Evidence of autonomy of aldosterone secretion, i.e. little or no response to salt loading, salt restriction or volume expansion, strongly support the diagnosis of primary aldosteronism due to an aldosterone-producing tumor. However, aldosterone secretion is not always completely autonomous in primary aldosteronism. Finally, primary aldosteronism has to be differentiated from Bartter's syndrome (1962, see secondary aldosteronism), i.e. juxta-glomerular hyperplasia, which is thought to be due to an extremely decreased vascular responsiveness to angiotensin II, leading to renin excess and secondary aldosteronism without hypertension.

In the rare syndrome of 17-hydroxylase deficiency, the clinical features of hypermineralocorticoidism are associated with signs of hypogonadism (amenorrhea, absence of secondary sex characteristics). Aldosterone secretion rate is decreased, but urinary and plasma 17-hydroxycorticoids and 17-ketosteroids are also very low. In this disease, as in ACTH-dependent secondary aldosteronism, potassium balance and blood pressure become normal under suppressive therapy with low doses of glucocorticoids. Primary aldosteronism has to be distinguished from Cushing's syndrome, which, however, is mainly characterized by the classic features of glucocorticoid excess, whereas hypokalemia is usually moderate. Cushing's syndrome due to ectopic secretion of ACTH by a malignant tumor, however, can be very similar clinically to Conn's syndrome, because hypokalemia is very pronounced and the classic Cushing's signs may be absent. But aldosterone excretion and secretion rate are usually normal or decreased, whereas the urinary cortisol metabolites, such as 17-hydroxycorticoids, are increased in Cushing's syndrome, in contrast to Conn's syndrome.

The diagnosis of primary aldosteronism is difficult, since there are no definite pathognomonic findings. Frequently, a high aldosterone excretion rate due to other factors leads to the wrong diagnosis of primary aldosteronism, even when the estimation has been carried out in the correct conditions with a diet containing at least 6 g of sodium chloride. The differential diagnosis between Conn's syndrome and malignant or renal hypertension is the most difficult. It should always be carried out at a specialized clinical center with adequate laboratory facilities.

A suspected diagnosis of primary aldosteronism should always be substantiated by means of electrolyte balance studies before expensive and time-consuming aldosterone, renin, and angiotensin estimations are carried out.

η) Therapy

When the diagnosis of primary aldosteronism is supported by the classic clinical features and has been confirmed by laboratory findings, and when malignant hypertension and unilateral kidney disease have been excluded, exploratory surgery of the adrenals is indicated. As a rule, substitution therapy with cortisol is not necessary, but it should always be prepared. If an adenoma is found the affected adrenal gland must be removed. Exploration of the opposite side can be omitted, because the tumor is unilateral in 98% of all cases. It is advisable to restore body potassium as far as possible by preoperative treatment with potassium, 200 mEq per day, spironolactone for 7 to 10 days, and sodium restriction. When surgery is contraindicated, spironolactone can be used for chronic therapy. The effectiveness of inhibitors of aldosterone biosynthesis, such as SU 9055 and aminoglutethimide, in the definite or preoperative treatment of primary aldosteronism has not been definitely evaluated. The heparinoid RO 18307 is not yet available for clinical use.

After removal of an aldosterone-producing adrenal adenoma, the metabolic syndrome with hypokalemia disappears within a few days. The blood pressure returns to normal within a few months in two-thirds of cases; hypertension is alleviated in 20% and unchanged in 13% of the patients.

b) Secondary Aldosteronism

α) Definition and Pathogenesis

The term "secondary aldosteronism" refers to increased aldosterone secretion due to extraadrenal causes. In most cases, elevated renin and angiotensin production is responsible for the increase in aldosterone secretion. In diseases with edema, increased renin production is a normal renal response to the decreased circulating blood volume. In cases of malignant renal hypertension, however, in response to an impaired renal circulation, it is pathologic. Hypersecretion of aldosterone alone does not lead to edema. Other factors, such as reduced oncotic pressure or abnormal capillary permeability, must have a primary role in the formation of edema. Aldosteronism in the 3rd trimester of a normal pregnancy is probably physiological and compensates for the sodium diuretic action of progesterone excess.

Since methods for measuring renin concentration or activity in the plasma have become available a few cases of what appears to be secondary aldosteronism have been described, in which renin-angiotensin production is not increased and in which another stimulatory mechanism had to be sought for the elevated aldosterone secretion rate. In some of these cases, the increased aldosterone secretion appeared to be dependent on ACTH. In other cases, the pathogenesis was completely unknown.

β) Aldosteronism Secondary to Edema

1. Nephrotic Syndrome. In the nephrotic syndrome of any etiology, oncotic pressure decreases due to renal loss of albumin, thus facilitating a shift of water and sodium chloride from the capillaries to the interstitial space and impairing

the reentry of these substances into the blood stream. Hypovolemia develops, which is a physiological stimulus of aldosterone production. Aldosterone secretion and excretion rates are usually markedly increased and may reach excessive values. More sodium is reabsorbed in the distal tubules, which further increases the interstitial fluid volume, setting up a vicious circle. It is still not known why this type of secondary aldosteronism does not lead to hypokalemia.

2. *Liver Cirrhosis.* Secondary aldosteronism is usually found in liver cirrhosis with ascites. Transudation from the blood stream leads to hypovolemia, which induces an increased secretion of renin and aldosterone. Moreover, inactivation of aldosterone by the liver is impaired, resulting in high values of urinary free aldosterone and 18-glucuronide, and a relatively decreased excretion of tetrahydroaldosterone.

3. *Idiopathic Edema.* Generalized edema not caused by renal, cardiac, or liver disease and occurring exclusively in women, usually in younger age groups and often characterized by premenstrual exacerbation, must be primarily due to an increased capillary permeability. Aldosterone excretion and secretion rates are not suppressed by intravenous infusion of sodium chloride. Sodium administration does not expand the intravascular volume, since the sodium immediately escapes into the interstitial space. Hypovolemia provides the stimulus for the increased production of aldosterone, occasionally leading to the formation of adrenocortical adenomas. In very severe cases leading to invalidity, subtotal or total adrenalectomy has been attempted. Idiopathic edema is related to cyclic edema, which occurs premenstrually in nervous women. There are intermediate forms of the two conditions. In some cases, occurrence of abnormal proteins has been demonstrated (THORN, 1968; VEYRAT, 1968; GILL, 1972).

4. *Heart Failure.* Cardiac insufficiency is usually accompanied by sodium retention and edema formation. Aldosterone excretion rate is often but not always increased. The secretion rate is normal in most cases. The role of aldosterone in edema formation of cardiac failure is not considered to be very important. The increased renal retention of sodium is thought to be due mainly to hemodynamic factors because renal sodium retention is promoted to a greater extent by anoxemia than by renal ischemia. Since aldosterone secretion rate is usually normal, the existence of aldosteronism secondary to cardiac failure is no longer unanimously accepted. The paradoxical reactions of cardiac patients to sodium restriction or loading are striking. Sodium restriction leads to disappearance of the edema, alleviates heart failure, and decreases aldosterone excretion. On the other hand, sodium loading in preexisting hypovolemia leads to a further expansion of the interstitial volume, increases edema, aggravates the cardiac failure, and induces an additional increase in aldosterone secretion. In heart failure, an inadequate renal blood flow (forward failure) leads to increased renal salt retention, whereas an increased venous pressure (backward failure) has the same effect on the kidneys by diminishing their oxygen supply.

γ) Aldosteronism Secondary to Malignant or Renovascular Hypertension with Renin Excess

Contrary to previous reports, aldosterone secretion or excretion rate is not increased in uncomplicated essential hypertension. However, in hypertensives the response to sodium restriction or sodium loading is blunted. It is still not known whether the diurnal variation in aldosterone secretion is greater in hypertensive patients than in normal healthy subjects.

Complications occurring in the course of essential hypertension, such as heart failure, renal insufficiency, or disorders of cerebral circulation, occasionally lead to an increased aldosterone secretion rate.

Malignant hypertension, which is always rapidly progressive and is characterized by high diastolic blood pressure, papilledema and renal damage due to necrotizing arteriolitis, is almost always associated with a strikingly increased aldosterone secretion rate. Despite manifest renal failure, there is a tendency to develop hypokalemia and alkalosis, so that primary aldosteronism is simulated. In contrast to primary aldosteronism, however, hypernatremia is never found and the serum sodium may even be slightly decreased. In malignant hypertension, aldosterone secretion is presumably stimulated by the renin-angiotensin system.

Excessive aldosterone production is often, but not regularly found in unilateral kidney diseases, particularly in stenosis of the renal artery with frequently malignant hypertension. These diseases are often associated with hypokalemia, but never with hypernatremia.

After surgical removal of the affected kidney, hypertension regresses and aldosterone secretion becomes normal. The increased aldosterone secretion rate is most probably mediated by the renin-angiotensin system. This condition may be mistaken for primary aldosteronism and the differential diagnosis has to be based on the

following tests: intravenous pyelography, Howard's test (comparison of sodium excretion on both sides), aortography, and where possible, determination of renin or angiotensin in peripheral blood, preferably in blood from the right and left renal veins, obtained by venous catheterization.

A few cases of a peculiar illness in which hypokalemia, polyuria and alkalosis are present but not hypertension ("Bartter's syndrome") have been described by BARTTER (1962) and other clinical investigators. This condition was at first confused with CONN's syndrome. The histology of the kidneys revealed hyperplasia of the renin-producing juxta-glomerular cells. BARTTER assumed that the disorder was primarily due to a failure of the blood vessels to respond to angiotensin, which was demonstrable by means of an angiotensin infusion test. This would lead to overproduction of renin and angiotensin, which in turn would stimulate aldosterone secretion and generate secondary aldosteronism. Hypernatremia is not found and the serum sodium may even be decreased. Some authors have suggested that the primary disorder might be renal salt loss or an anomaly in the regulation of extracellular fluid volume (BEILIN, 1967). Similar cases have been observed in association with growth retardation. Some cases may previously have been mistaken for primary aldosteronism with bilateral adrenal hyperplasia.

δ) Glucocorticoid-Suppressible Secondary Aldosteronism

SUTHERLAND (1966) first described a disease which occurred simultaneously in father and son and was characterized by benign hypertension, potassium deficit, increased plasma volume, elevated aldosterone secretion rate, intermittent elevation of urinary 17-hydroxycorticoids and 17-ketosteroids, normal secretion rate of corticosterone and compound S, normal plasma ACTH and low plasma renin activity. Surgery in the father revealed no adrenal tumors. Treatment with dexamethasone, 2 mg per day, led to marked suppression of aldosterone secretion and normalization of blood pressure and serum potassium in father and son. A similar disease was observed by NEW (1967) in a 12-year-old boy. He had a moderately elevated aldosterone secretion rate, reduced plasma renin activity, elevated plasma ACTH concentration and low normal values or urinary 17-ketosteroids and 17-hydroxycorticosteroids with a delayed response to ACTH. Treatment with prednisone in a dose of 10 mg daily suppressed aldosterone secretion and normalized blood pressure and serum potassium.

ε) Secondary Aldosteronism of Unknown Etiology ("Primary Aldosteronism with Bilateral Adrenal Hyperplasia", "Pseudo-Primary Aldosteronism", "Idiopathic Aldosteronism")

Since the determination of plasma renin concentration or activity was introduced as a diagnostic tool for the detection of primary aldosteronism, several cases have been described in which a preoperative diagnosis of primary aldosteronism was based on an increased aldosterone secretion rate and a simultaneous suppression of plasma renin activity and surgery did not reveal a tumor but bilateral adrenal hyperplasia. It must be assumed that an unknown stimulatory principle was responsible for the increased aldosterone secretion rate and the adrenocortical hyperplasia (DAVIS, 1967; SALTI, 1969). This disease occurs almost as frequently as primary aldosteronism due to an aldosterone-producing adrenal adenoma (BAER, 1970). Hypertension is generally not improved by subtotal or total bilateral adrenalectomy. A definite preoperative differential diagnosis between this disease and primary aldosteronism can only be made by the determination of aldosterone in adrenal venous plasma obtained from both sides by catheterization.

ζ) Therapy of Secondary Aldosteronism

Treatment of secondary aldosteronism consists primarily in removing the cause, i.e. treatment of the underlying disease. Occasionally, treatment with aldosterone antagonists, which act on the target organs of aldosterone, or with inhibitors of aldosterone biosynthesis can be helpful. SU 9055, a specific inhibitor of aldosterone biosynthesis, in a daily dose of 4.8 g reduces the elevated aldosterone secretion rate to very low levels and promotes sodium excretion in the urine. The clinical value of this drug has not yet been definitely assessed.

c) Excess of Other Mineralocorticoids

α) 17-Hydroxylase Deficiency

This rare disease is a congenital disorder of corticosteroid biosynthesis. However, it is not characterized by an adrenogenital syndrome, but rather by hypogonadism and hypermineralocorticoidism. Less than 10 cases have been described. Most of these patients have been young women with primary amenorrhea, lack of secondary sexual characteristics, hyperten-

sion, hypokalemia, and metabolic alkalosis. In boys, the disease is characterized by pseudohermaphroditism (NEW, 1970). The secretion of aldosterone, cortisol, androgens, and estrogens is strikingly decreased, while the production of corticosterone, deoxycorticosterone and progesterone is increased. Plasma renin activity is very low, while levels of ACTH, LH and FSH are elevated. Suppressive therapy with dexamethasone (1 to 2 mg per day) or with cortisone decreases corticosterone and deoxycorticosterone production and leads to normalization of blood pressure and potassium balance.

β) Deoxycorticosterone Excess

A case of hypermineralocorticoidism due to a pathologically increased deoxycorticosterone secretion rate with a normal aldosterone secretion rate has been described by BIGLIERI. The 39-year-old female patient had edema, hypovolemia, hypotension, and hypokalemic alkalosis. A bilateral nodular adrenal hyperplasia was found at operation.

γ) 18-Hydroxydeoxycorticosterone Excess

An increased secretion rate of 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) or an elevated urinary excretion rate of tetrahydro-18-OH-DOC was found by MELBY (1972) in patients with benign hypertension, low plasma renin and normal or slightly decreased aldosterone secretion rate. Some of these patients became normotensive under suppressive therapy with dexamethasone (1.5 mg per day).

3. Cushing's Syndrome

a) Definition

The typical clinical features of Cushing's syndrome are always due to an excess of endogenous cortisol or exogenous glucocorticoids. There are various causes of the disease (see below).

The differentiation between Cushing's syndrome without specification of the etiology and Cushing's disease due to a basophilic pituitary adenoma is no longer justified, since the existence of an autonomous basophilic adenoma of the pituitary gland has become questionable.

b) Incidence

Cushing's syndrome is a rare disease. PLOTZ (1952) reported 189 cases, verified by autopsy or hormone determinations, from the literature, SOFFER (1961) 450 cases. At the Department of Medicine of the University Hospital of Zurich, 30 cases of Cushing's syndrome have occurred among 30000 patients within the last 10 years. In the Zurich Children's Hospital, 6 cases were observed among 70000 patients. The syndrome has been observed 15 times in 33000 autopsies carried out during the last 20 years at the Pathology Institute of the University of Zurich. The syndrome is 3 to 4 times more common in women than in men, and often appears following pregnancy. Its incidence is greatest in the 3rd and 4th decades, but it may occur at any age, from earliest childhood (youngest case 4 months old) to senescence.

c) Pathogenesis and Etiology, Pathophysiology

Cushing's syndrome is always caused by an overproduction of steroids with glucocorticoid activity, which are almost exclusively formed in adrenocortical tissue. In some instances, cortisol has been reported to originate from ovarian and testicular tumors which were probably derived from detached portions of adrenocortical tissue.

Glucocorticoid excess can result from:

1. Primary adrenal lesion (adenoma, carcinoma);
2. A disorder of the hypothalamic-pituitary mechanism regulating adrenocortical activity (bilateral hyperplasia);
3. Excessive stimulation of the adrenals by ACTH produced by ectopic tumors (bilateral hyperplasia);
4. Excessive administration of exogenous cortisol or its derivatives (adrenal cortical atrophy) or of ACTH (bilateral hyperplasia).

1. Adenomas (15%) and carcinomas (10%) of the adrenal cortex, and in a few cases of detached adrenocortical tissue in the ovaries and testes, usually grow autonomously, i.e. independently of pituitary control, in the same manner as other benign or malignant tumors. Their steroid production is also autonomous to a great extent and therefore not suppressible in the 8 mg dexamethasone test. The cortisol production of adrenal adenomas can occasionally be stimulated by exogenous ACTH, but only to a small extent, whereas carcinomas are usually not responsive. The cortisol secreted by the tumor suppresses the endogenous ACTH secretion, and thus leads to atrophy of both the ipsilateral and the contralateral adrenal glands. Normal or hyperplastic adrenal glands with small multiple adenomas found at operation indicate that the disease is most probably due to hypothalamic-pituitary factors.

2. Cushing's syndrome with adrenocortical hyperplasia is due to excessive ACTH production by the anterior pituitary gland. Small, or less commonly large, basophilic or chromo-

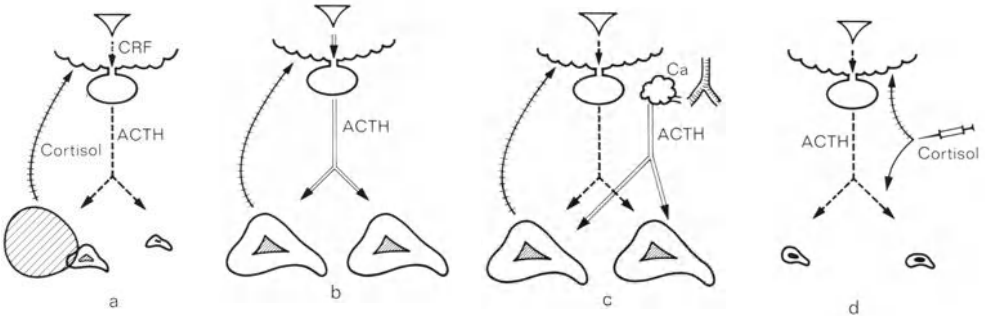


Fig. 18a–d. Causes of Cushing's syndrome. a) Adrenocortical tumor; b) Adrenocortical hyperplasia of hypothalamic origin; c) Adrenocortical hyperplasia due to ectopic ACTH production; d) Exogenous cortisol administration

phobe adenomas may, but need not be found. Occasional invasive extracellular growth of these tumors can lead to lesions of cerebral nerves, in particular of the oculomotor nerve. The existence of autonomous pituitary tumors producing excessive ACTH in proportion to their size is no longer accepted. Since it has been known that these tumors develop particularly often after total adrenalectomy, i.e. after removal of the suppressive influence of cortisol, they have been considered to be hyperplasiogenic, developing under increased stimulation by the hypothalamic corticotropin-releasing factor. Cushing's syndrome with bilateral adrenal hyperplasia is thus due to impaired regulation of ACTH secretion. The hypothalamus is less sensitive to the negative feedback effect of cortisol, i.e. the regulatory circle is set to a higher cortisol level (Fig. 19). Cortisol exerts its suppressive effect on the hypothalamus and the pituitary gland only at higher concentrations.

LIDDLE (1960, 1962) has substantiated this hypothesis by the determination of ACTH in human plasma and by assessing its suppressibility by various concentrations of cortisol and dexamethasone, and has at the same time introduced the dexamethasone suppression tests (see p. 389), which are the most reliable tools currently available for the diagnosis and differential diagnosis of Cushing's syndrome. Whereas in healthy subjects the plasma ACTH concentration varies between 0.1 and 0.5 mU per 100 ml and is thus just measurable by the most sensitive bioassays, it increases 10- to 100-fold in Addisonian patients and returns to the normal range after substitution with cortisol, 30 mg per day. Plasma corticotropin concentration in untreated Cushing's syndrome is slightly elevated or in the upper normal range (NELSON, 1966), since a certain, although insufficient suppression of ACTH release is exerted by the markedly elevated cortisol concentration in the plasma. The diurnal variation is lost, and the absence of the nocturnal fall in ACTH secretion leads to a twofold increase in the daily cortisol output (RETIENE, 1965). After total adrenalectomy in a patient with Cushing's syndrome and under normal substitution with 30 mg of cortisol per day, the ACTH concentration increases markedly. The corticotropin concentration only regresses to normal values when the dose of cortisol is increased several times (8-fold) (Fig. 19).

Thus, therapeutic adrenalectomy and normal replacement therapy in patients with Cushing's

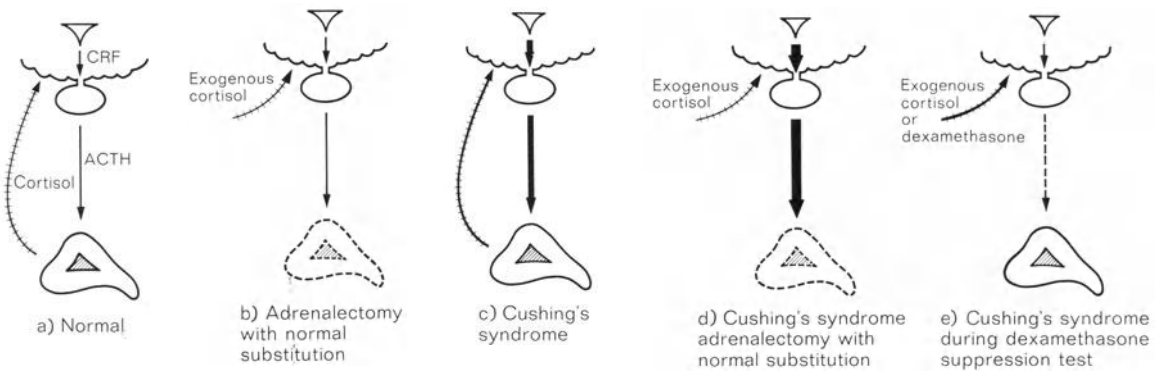


Fig. 19a–e. The hypothalamic-pituitary control of adrenocortical function in normal subjects, in patients after adrenalectomy and in Cushing's syndrome. a) Normal; b) Adrenalectomy with normal substitution therapy; c) Cushing's syndrome; d) Cushing's syndrome after adrenalectomy with normal substitution therapy; e) Dexamethasone suppression test in Cushing's syndrome

syndrome lead to a persistent increase in ACTH secretion, clinically manifested by a striking increase in pigmentation and a tendency to adenohypophyseal adenomas. Pituitary adenomas occurring in patients with Cushing's syndrome after therapeutic adrenalectomy have been observed in dozens of cases. The plasma ACTH concentration is extremely high in these patients, and values of up to 1000 mU per 100 ml have been determined. The circadian rhythm is absent at first, but reappears at an elevated level.

3. Peptides with ACTH activity can be produced outside of the pituitary gland by neoplastic tissue, particularly by bronchial carcinomas and their metastases. As in the second type of Cushing's syndrome, bilateral hyperplasia of the adrenal cortex develops. Strikingly elevated ACTH levels are found in the plasma. These tumors are not affected by feedback inhibition and their ACTH production is not suppressed by cortisol or dexamethasone. Very high ACTH levels and the complete absence of suppression indicate ectopic ACTH production (NELSON, 1966).

4. Excessive administration of cortisol and its derivatives or ACTH for therapeutic purposes leads to the iatrogenic Cushing's syndrome, which differs very little from endogenous cortisol excess.

Hirsutism and acne are more common in iatrogenic Cushing's syndrome due to ACTH administration than during treatment with high doses of corticosteroids.

Cushing's syndrome may exceptionally develop during maintenance therapy with normal doses of cortisone (25 mg per day) when metabolic degradation is impaired, e.g. in secondary hypothyroidism.

Every case of Cushing's syndrome (with the exception of the iatrogenic type) is due to increased cortisol production. The cortisol secretion rate shows a 2- to 3-fold increase. By contrast, the secretion rate of corticosterone is usually not elevated, or only slightly. However, markedly increased corticosterone production has been observed in cases of adrenal carcinoma and in the ectopic ACTH syndrome. The secretion rate of aldosterone is generally normal or decreased (BIGLIERI, 1963; GUINET, 1967).

The metabolic activity of cortisol (see p. 303), i.e. the promotion of gluconeogenesis, leads to a negative nitrogen balance, a tendency to high blood sugar levels, and a pathological result in the glucose tolerance test, which can initially be partly compensated by an increased insulin secretion. The normally minimal glucosuria is increased due to accelerated glucose filtration. On the other hand, peripheral carbohydrate metabolism is not impaired in pure steroid diabetes. Ketosis does not occur and the liver glycogen is increased. The findings of elevated plasma levels of pyruvic acid and lactic acid (HENNEMANN, 1957) may possibly be related to the increased supply of substrates provided by gluconeogenesis. Manifest diabetes with reduced glucose assimilation in the course of Cushing's syndrome probably only occurs in individuals with a genetic predisposition for diabetes. Treatment with cortisone can be used as a screening test for diabetes (see p. 813f.).

d) Pathologic Anatomy

α) Hyperplasia of the Adrenal Cortex

In cortical hyperplasia, both adrenals are usually enlarged but maintain their shape. Enlargement often appears to be trivial. In the older statistics of the weight of normal adrenal glands, too little attention was paid to adrenocortical changes during adaptation to stress and diseases. The allegedly normal values given are therefore too high. If only glands from healthy subjects who have died suddenly and violently are considered, the normal average weight of both adrenals of adults is 10 g, with a range of 6 to 11 g. Thus, a combined weight of 10 g is compatible with adrenocortical hyperplasia.

In *simple hyperplasia*, the cortical structure remains intact, but definite changes in the relative and absolute width of the individual zones are observed.

Since hyperplasia is usually not quite uniform, zones of increased proliferation develop, leading to *nodular hyperplasia*. These nodules can be situated in the cortical tissue itself and are frequently not clearly circumscribed. In the capsular region, they are often sequestered from the cortical tissue and become encapsulated, which leads to a nodular surface of the adrenal. As in other endocrine organs, particularly in the anterior pituitary gland and the thyroid, the transition from nodular hyperplasia to adenomas is gradual.

β) Bilateral Adrenocortical Adenomatosis and Adenomas

The differentiation between nodular hyperplasia and adenomas is particularly difficult in *bilateral adrenocortical adenomatosis* of Cushing's syndrome or adrenogenital syndrome. In typical cases, adrenocortical areas not affected by nodular hyperplasia become atrophic. Adenomas are usually solitary. According to a review by RAPAPORT (Table 12), both adrenals are affected with approximately the same frequency. Bilateral tumors occur in 1 to 2% of cases. In almost

Table 12. Classification of adrenocortical tumors of 278 patients according to their endocrine activity. [After a review by E. RAPAPORT, M. B. GOLDBERG, G. S. GORDAN, and F. HINMAN JR.: Postgrad. Med. **11**, 325 (1952). The figures refer to the statements made in the text which do not quite agree with those in the tables]

	Number of cases	% of all cases	% of the tumors with hormonal activity only
1. Hormonally inactive tumors	43	15.5	–
2. Tumors with androgenic activity	80	28.8	34.0
3. Tumors with Cushing's syndrome	122	43.9	51.9
4. Tumors with estrogenic activity	7	2.5	3.0
5. Tumors with estrogenic and androgenic activity	4	1.4	1.7
6. Tumors with hypoglycemia	3	1.1	1.3
7. Choriongonadotropin-producing tumors	2	0.7	0.9
8. Non-classifiable tumors (insufficient information)	17	6.1	7.2
Total	278	100.0	100.0

2% of the cases observed the tumors have originated from aberrant adrenocortical tissue located near the adrenals and the kidneys, in the retroperitoneal space distal to these organs, and in the genital region (see adrenal dystopia).

Many adenomas do not lead to clinical manifestations and they are found at autopsy more frequently than expected.

Some adrenocortical adenomas become very large and reach a diameter of several cm and a weight of 100 to 200 g, occasionally even of kg. In 20% of cases, the tumors become manifest because of their size and are sometimes palpable. The adenomas are usually round and firmly encapsulated. In contrast to nodular hyperplasia, the adjacent cortical tissue is often compressed; cut surfaces are yellowish brown, lobular and sometimes penetrated by hemorrhage and cysts. Histology of the adenomas shows that they are composed of nests of cells similar in appearance to different layers of the normal cortex. Cells are usually large and often contain lipids and glycogen. The pigment, on the other hand, is not as abundant as in the zona reticularis. Occasional groups of spindle cells resembling pheochromocytoma tissue may be present.

γ) Carcinomas

Adrenocortical carcinomas may be very similar to adenomas both microscopically and macroscopically. Occasional invasion of the capsule and penetrations into the veins can be seen with the naked eye, as in hypernephroid renal carcinoma. Cones of the tumor may reach the vena cava, and this must be borne in mind by the surgeon attempting radical exstirpation. Necrosis and hemorrhage are not uncommon. Microscopic diagnosis is not difficult when the tumors are poorly differentiated and have the characteristic features of carcinoma. However, if the tumors are more differentiated, distinction between adenomas and carcinomas may be very difficult. The structure of carcinomas may be very uniform, and adenomas may be characterized by cellular and nuclear polymorphia due to degenerative changes. In border-line cases, a decision is only possible on the basis of metastases.

The morphology of adrenocortical tumors does not allow any definite conclusions about their endocrine activity. Thus, the histological structure of hormone-inactive tumors may be absolutely identical to that of hormone-active adrenal cortical tumors producing different steroids and leading to corresponding clinical syndromes. Fuchsinophilia according to VINES, and other staining reactions such as Ashbel-Seligman's reaction, are not specific for androgens (BACHMANN, LIEBEGOTT). Apart from the morphologically detectable secondary symptoms, certain conclusions can be drawn from the pathologic anatomical nature of the remaining adrenal tissue. The state of the contralateral adrenal is of special significance, because an atrophy of the ipsilateral adrenal cortex may be due to pressure. Atrophy of the contralateral adrenal cortex caused by suppression of pituitary ACTH secretion is found in more than 70% of cases of Cushing's syndrome due to an adrenocortical adenoma or carcinoma. Contralateral adrenocortical atrophy is observed in approximately 30% of cases with androgen-producing tumors, but never in cases of hormone-inactive tumors.

δ) Metastases

Adrenocortical metastases are common. Only in rare instances do they lead to endocrine failure, and the clinical picture is usually dominated by the manifestations of the metastasizing tumor.

For a discussion ectopic hormone production and Cushing's syndrome see p. 353.

ε) Changes in the Pituitary Gland

Changes in the pituitary gland in Cushing's syndrome are very variable. Only Crooke cells, which can be demonstrated in a large proportion of cases, are characteristic. THOMPSON and EISENHARDT found them in the pituitary glands of 58 out of 63 patients with Cushing's syndrome. They are basophil cells with a peculiar hyalinization of the cytoplasma, with a tape-like hyalinized zone replacing the basophil granules (Fig. 20). The reader is referred to the reviews by PLOTZ (1952) and ROVIT (1969) for discussions of the incidence of pituitary changes.

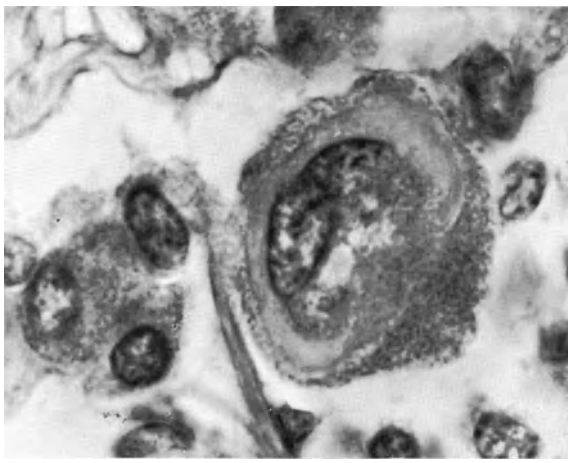


Fig. 20. CROOKE's cell in the anterior pituitary gland in Cushing's disease with anterior pituitary mucoid cell adenoma and bilateral adrenocortical hyperplasia. In comparison with a normal basophilic cell (left side) cytoplasma and the nucleus are enlarged. The perinuclear cytoplasma shows hyaline transformation. The hyaline mass is not stained by periodide leucofuchsin. Marginal remains of granulose cytoplasma. SN 321/55, 32-year-old women. Periodate leucofuchsin orange G stain according to PEARSE, enlargement 1137:1

The multiplicity of the hypophyseal changes becomes understandable when the transformational capacity of the adeno-hypophyseal cells and the different possible pathogeneses of Cushing's syndrome are considered. Basophil and large chromophobe cells, at least the so-called sparsely granulated cells, may correspond to the same type of cells under different functional conditions. Thus it is understandable that in Cushing's syndrome basophil, chromophobe and mixed cell adenomas are found, which may also metastasize in exceptional cases (ROVIT, 1969).

e) Clinical Features

α) History

When taking the case history, the doctor should try to fix the onset of the disease to a certain time. There is always some discontinuity in the life history in Cushing's syndrome. Physical and mental alterations may develop slowly without alarming the patient; but they can also appear quite suddenly, and the patient and his family may become aware of his altered appearance within weeks or days. The patient is always able to state a time at which he had not yet observed the changes. Earlier photographs can be particularly informative, as in numerous other endocrine diseases. The differentiation of Cushing's syndrome from constitutional obesity, which is often associated with hypertension and diabetes, is usually possible just from the history, since constitutional obesity appears in childhood and no striking changes are observed by the patient and his family.

The patients' main complaint is fatigue, which contrasts with the flourishing appearance. Fatigue can progress to adynamia and may finally cause complete confinement to bed. It is of the organic type, i.e. less pronounced in the morning than in the evening.

Back pain with radiation along the costal arch is characteristic of osteoporosis (see p. 349). Kidney-stone or gall-stone colics can occasionally be the first symptoms of the disease: increased calcium excretion promotes calculus formation. When diabetes mellitus becomes severe, the patients may be troubled with thirst, polyuria, and pruritus.

Mental Changes. Mental changes typical of endocrine psychosyndrome usually occur in manifest Cushing's syndrome. Apathia and excitement are frequent. Sexuality is often subdued; in rare cases it may be temporarily increased. Hunger and thirst are usually increased. Acute variations in mood can progress to episodes of psychosis in the patient with Cushing's syndrome. Confusion, hallucination, illusions, and delusional ideas may then occur. Although these psychoses can be very similar to schizophrenia, they must be considered as increases in the variation of mood and drive with progression into the acute exogenous reaction type. Amnestic symptoms are common in Cushing's syndrome. Mental disturbances are not correlated to physical changes. However, the psychopathology of a patient with Cushing's syndrome is closely related to the premorbid personality, and the mental alterations may often be an exaggeration of preexisting peculiarities.

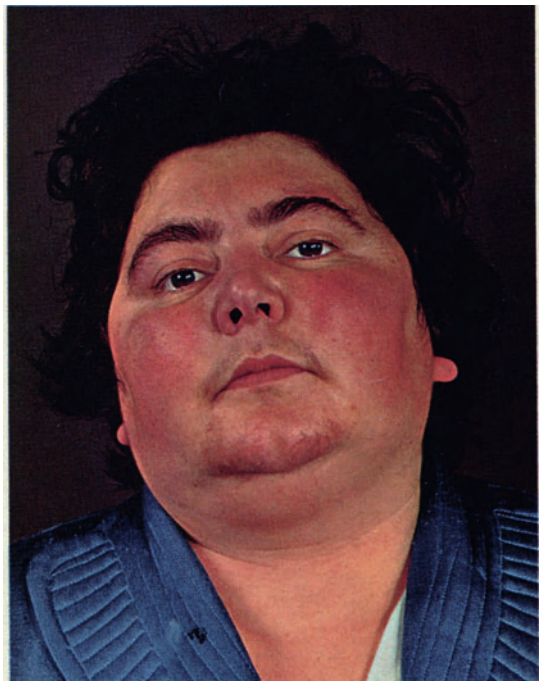


Fig. 21. 31-year-old patient with Cushing's disease due to a basophilic pituitary adenoma. Moon face, rubeosis, acne and moderate hirsutism. Low frontal hair border

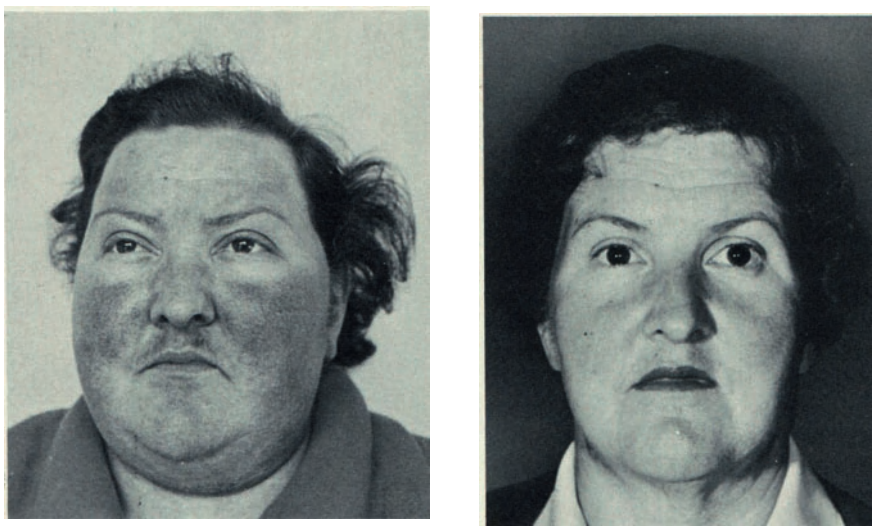
β) Physical Examination

There is hardly any other illness as characteristic, impressive, and unforgettable as Cushing's syndrome. The disfigurement of a beautiful and expressive face to ridiculous ugliness by this disease can be a shock to both patient and doctor. The experience of watching the face, characterized by the features of the patient's personality, reemerge from behind the mask of the disease after successful therapy can be just as impressive (Figs. 21–25). Briefly, the nature of the disease is an alteration in metabolism from protein synthesis to excessive glucose production, and thus indirectly to increased fat deposition. This leads to the 7 cardinal signs of Cushing's syndrome (Table 13):

Table 13. The 7 cardinal clinical manifestations of Cushing's syndrome

	Frequency (%)
1. Red, rounded face (plethora, moon face)	90
2. Truncal obesity	85
3. Decreased glucose tolerance-diabetes mellitus	85
4. Hypertension	80
5. Osteoporosis	70
6. Amenorrhea, hypogonadism	70
7. Purple striae, ecchymosis	60

Obesity of the Trunk. Muscles and bones regress and excessive adipose tissue is formed. Obesity of the trunk results, in which the extremities remain thin, but the head, neck, and body, where most of the storage fat is deposited, become thick and unshapely. The peculiarity of this fat distribution cannot be explained by the metabolism. The characteristic body shape of an endocrine disease is due to variable responsiveness of the tissues to a certain hormone. Increased fat deposition in the cheeks



a b

Fig. 22. a) Very severe Cushing's syndrome with truncal obesity, intensely red moon face with teleangiectasias, increased beard and body hair, hypertension up to 200/130 mm Hg. b) The same patient one year after total adrenalectomy (Dr. BIRNSTIEL, Pflegerinnenschule, Zurich)

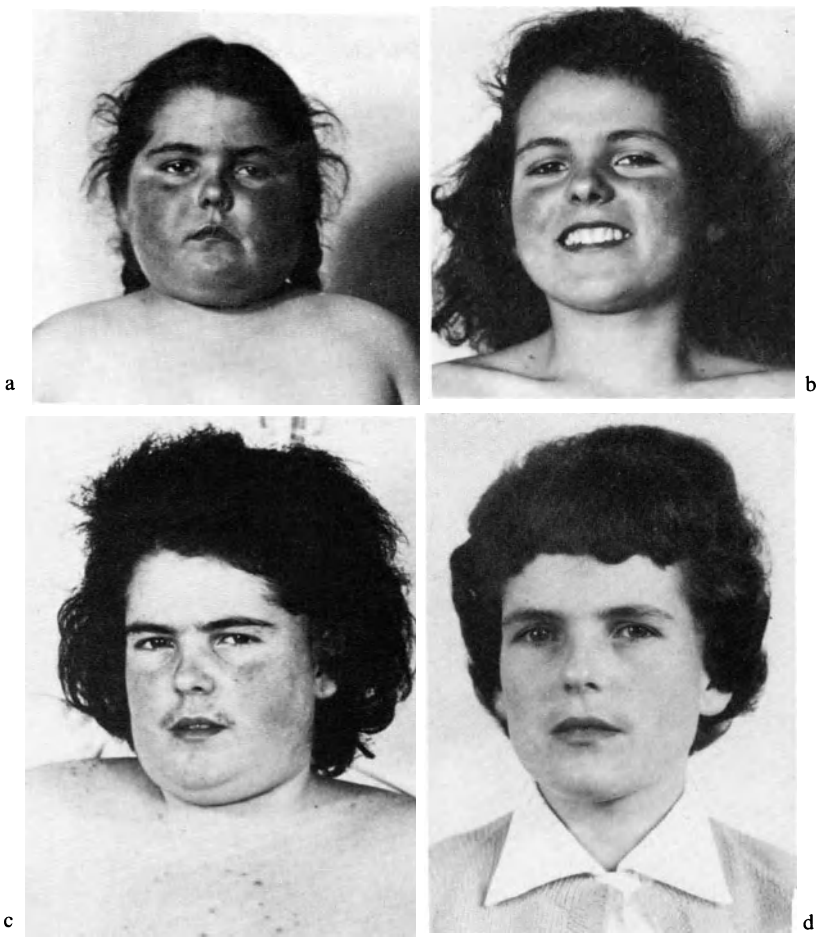


Fig. 23. a) 12-year-old girl with Cushing's syndrome. Arrest of growth and truncal obesity, hypertension 200/120 mm Hg. b) 10 months following X-ray irradiation of the pituitary with complete remission. c) Severe relapse with multiple spontaneous vertebral fractures 9 years later. d) The patient one year after total adrenalectomy (a and b KspZ)

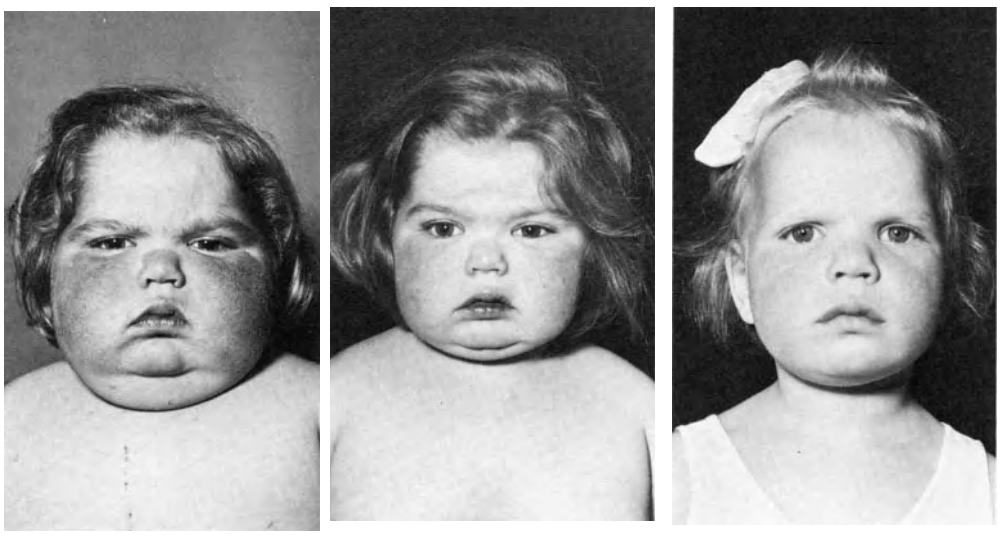
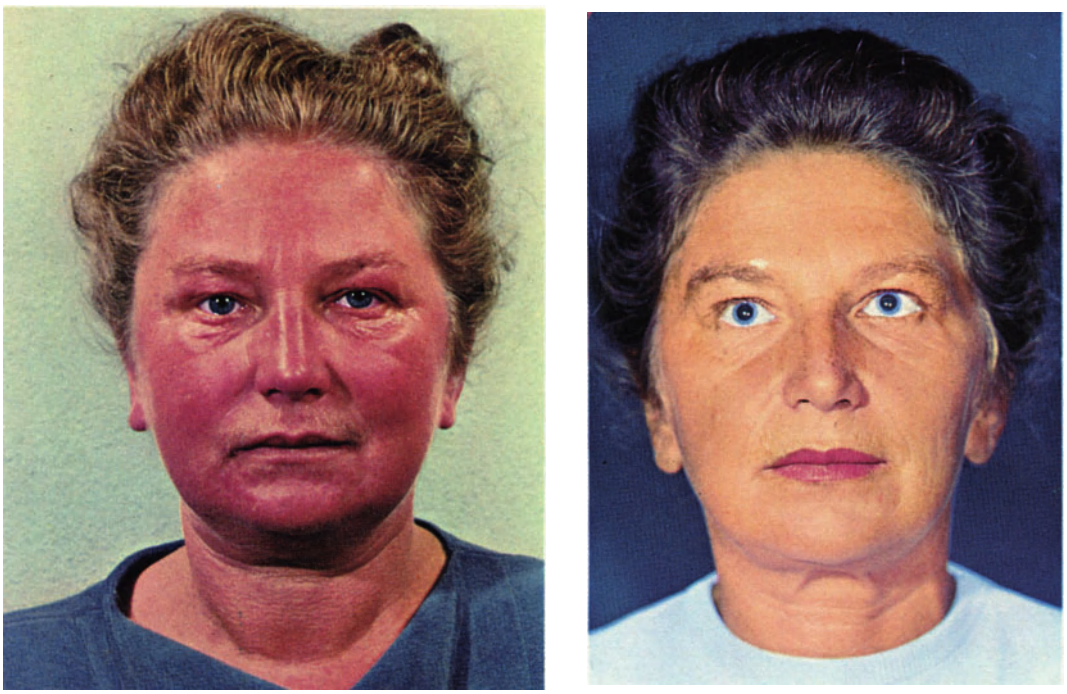


Fig. 24a–c. Alteration of facial expression in Cushing's syndrome. 4-year-old girl with Cushing's syndrome due to an adrenocortical carcinoma. a) Before removal of the tumor. b) One month after the operation. c) 7 months after the operation. (From GROB, PRADER, and ZOLLINGER)

leads to rounding of the face ("moon face") and also to slanting of the eyes and drooping of the corners of the mouth, which then resembles a carp's mouth. Simultaneous expressions of dignity and melancholia, often ridiculous by their contrast are often found in association with extreme forms of moon face, hiding the ears, in children. The striking redness and unnatural gleam of the skin leads to the impression of an unnatural doll's or clown's face (Figs. 21 and 24). Individual traits and sex specificity in the facial expression are suppressed.

The neck appears to be strikingly short, due partly to the collar-like fat deposit and the formation of a double chin, and partly to the pathologic increase of the physiological kyphosis of the thoracic vertebral column. The posture of the patient with Cushing's syndrome is characteristic in profile. The rounded head thrust forward and the short neck lend something threatening to the posture, in grotesque contrast to the portly stoutness of the body and the ugliness of the thin extremities. The panniculus adiposus of the lower abdomen is particularly developed, together with the flaccidity of the reduced abdominal muscles. Typical pendulous abdomen may appear, overhanging the genitalia like an apron. Adipose tissue is developed to a lesser extent in the region of the hips. The thighs are markedly conical. Excessive fat may also be deposited in the breasts, and the shoulders appear to be rounded and drooping. The angle between the neck and shoulder is filled out. An area of special predilection for fat deposit is the lower cervical spine, where a pillow-like fad pad may be formed, the so-called "buffalo hump" (JORES). The obesity may be severe but is not always so; excessive weight increase is not particularly typical of Cushing's syndrome. The total body weight may even be unaltered, so that only the unusual distribution of fat is conspicuous. The picture of flourishing health suggested by obesity and the rosy complexion is not always predominant. The muscular loss may be more impressive than the fat formation and suggest deterioration of the patient by the feebleness in posture. Replacement of the locomotor system by storage tissue is a process of aging, and premature aging is a further feature of this disease. Thus, Cushing's syndrome in a young woman leads to a caricature of the matronly type, and ALBRIGHT has in fact assumed that there is a relative predominance of the glucocorticoids over their anabolic antagonists in the interval between the menopause and the onset of senescence in the life of a woman (menopause-adrenopause). The



a b

Fig. 25. a) 42-year-old patient with slowly developing Cushing's syndrome due to an adrenocortical adenoma. No obesity, moderate rounding and marked redness of the face. b) The same patient 3 years after removal of the adrenocortical adenoma: without weight loss disappearance of the redness, altered facial expression

apparent similarities may thus be based on the corresponding metabolic states rather than be coincidental.

However, comparison with the matronly type does not give any impression of the inscrutable appearance evoked in patients with Cushing's syndrome by their tired and suffering expression combined with the disfiguring fullness of the body.

Reduction of the visual fields due to pituitary tumors, occurs in about 5% of cases (see p. 341). This is sometimes combined with paralysis of the ocular muscles. Whether the intraocular pressure is generally elevated and can lead to glaucoma (BAYER, 1967) has to be investigated in a larger number of patients.

In some cases, pronounced exophthalmus may be caused by increased retrobulbar adipose tissue. The etiology is unknown.

In women and children, all forms of Cushing's syndrome are accompanied by mild hirsutism in most instances. In its mildest form, it is a fine fluff on the upper lip and cheeks. Even in pure cortisol excess, the weakly androgenic cortisol metabolites may lead to hirsutism when they are present in large quantities. Acne is almost always present on the face and upper trunk (see Fig. 23). In pure Cushing's syndrome, the forehead hairline descends further forward, particularly in the lateral areas, where angular baldness due to androgen activity develops. The eyebrows also become bushy with lateral widening.

Skin. In Cushing's syndrome, the skin is thin, stretched, and shiny due to the effect of the fat. When the disease is of long duration, the skin becomes atrophic, as in old people. Its blood supply is ample. It is warm to the touch, oily, and moist. Pyodermia of the seborrhoic skin is frequent and heals poorly. The color of the skin, especially in the face, is a striking dark red, which is frequently described as "plethoric" or "congested". The remaining skin of the body may be mottled and the extremities are usually dark red to cyanotic. The plethoric appearance is not always compatible with the existing polycythemia. It must be due to an expansion of the capillaries and to a partial stasis. In addition to the plethoric tinge, there is increased pigmentation in a few cases, similar to that in Addison's disease, which may be due to the raised ACTH level or to increased MSH secretion by the pituitary gland. However, it never reaches the same severity as in Addison's disease. Violaceous striae are characteristic. Only in pregnancy are they similarly impressive. They are dark red to violet stripes running parallel to the tension lines of the skin. They are due to dilated subcutaneous venous plexuses and perhaps also to extravasates into the subcutaneous adipose tissue shimmering through the overlying stretched and slightly pitted epidermis (Fig. 26). They occur most frequently on the anterolateral parts of the lower abdomen, on the breasts and the thighs, and in the adipose tissue of the axilla. Although they are found only in Cushing's syndrome and in pregnancy in this intensity, they are by no means pathognomonic of these conditions. Any form of obesity, developed rapidly and especially during youth, can give rise to striae, but they are then usually pink rather than dark red and more numerous, but finer (see p. 354). They may also occur during puberty, even without simultaneous obesity.

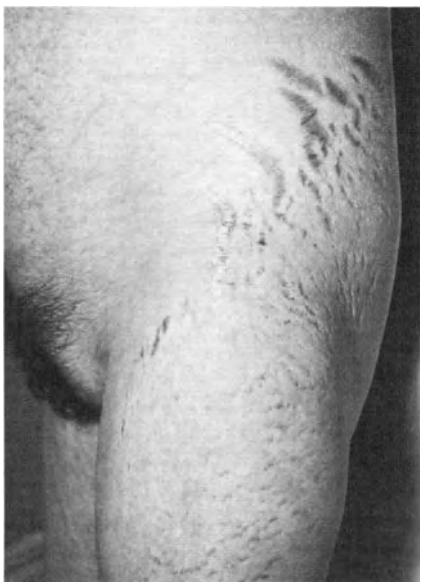


Fig. 26. Purple striae in Cushing's syndrome (KspZ)

The prediposition to hemorrhage is not only apparent in the violaceous striae, but also leads to the additional or exclusive formation of petechia and patchy suffusions, especially on the extensor sides of the forearms, but also in other areas of the body exposed to pressure. This may finally lead to the picture of hemorrhagic diathesis. In rare cases, hemorrhage into the intestinal lumen may occur, resulting in death. Generally, the skin is more bruisable, and slight injuries may result in extravasation of blood under the epidermis. Neither the striae nor the remaining adipose tissue are exceptionally tender. Edema occurs in approximately half the cases.

The pathogenesis of the cutaneous changes is assumed to be related to the antianabolic properties of cortisol. Violaceous striae can also

be provoked by exogenous ACTH and cortisol. Blood coagulation factors are unaltered. Bleeding time and coagulation time are always normal, whereas Rumpel-Leede's sign may be positive. Hemorrhage is thus due to vascular factors.

Hypertension. Raised blood pressure is an important observation in the general examination. It varies in severity. It rarely leads to the picture of malignant hypertension with nephrosclerosis, albuminuria, renal insufficiency, and apoplexy. Blood pressure values are often only slightly elevated. Apparently some other constitutional factor is necessary for the development of hypertension, since only some of the patients develop hypertension during treatment with high doses of cortisone. The pathogenesis of the hypertension is still unexplained. It must be due to the cortisol excess, because the secretion of mineralocorticoids is not increased. An increased supply of sodium to the arteriolar musculature may play some part, since cortisol impairs the renal sodium excretion. In addition, cortisol is essential for the hypertonic action of noradrenaline (permissive action, see p. 307).

Osteoporosis. Diffuse osteoporosis of varying severity is an important sign of CUSHING'S syndrome. Under the effects of cortisol, the transformation of bone is extensively retarded, with a preponderance of catabolism over anabolism (FROST, 1963). The calcium balance of the body becomes negative since enteral resorption is diminished, and calciuria results from decreased tubular reabsorption. Osteoporosis leads to static deformation of the vertebral bodies, occasionally combined with infractions. Spontaneous fractures of the ribs and vertebral bodies often occur, resulting in conspicuous proliferative callus formation. Wedge-shaped vertebrae may be formed in the thoracic section and fish vertebrae in the lumbar section of the spine. Demonstration of vertebral deformation confirms osteoporosis. It is extremely difficult to diagnose osteoporosis from the diminished shadow density of the bones alone. Osteoporosis is diagnosed much too frequently in corpulent patients whose X-ray pictures are poor in contrast. Demonstration of greatly accentuated borders of the vertebral body, which looks as if it had been drawn in by pencil, may be significant in the differentiation between osteoporosis and apparent radiolucency.

Muscular Atrophy. Atrophy of the muscles is due to the catabolic, antianabolic activity of cortisol. It can lead to complete invalidity, but is reversible by effective therapy. There is creatinuria and a negative nitrogen balance. Severe hypokalemia can contribute to adynamia, particularly in the paraneoplastic Cushing's syndrome.

Diabetes Mellitus. Diabetes mellitus is part of the fully developed Cushing's syndrome. Manifest diabetes is not common (15%); however a diabetic type of altered metabolism can be recognized by determination of blood sugar or a glucose tolerance test in almost every case. So-called "steroid diabetes", is a special type of diabetes mellitus, which is not due to insulin insufficiency, but to cortisol excess. As in the usual form of diabetes, too much glucose is formed, but in contrast to the usual form, peripheral utilization of glucose is normal. Steroid diabetes is benign at first with moderately elevated blood sugar values; the course is stable, and ketoacidosis never develops. Insulin, however, has little effect, and complications are not infrequent despite the apparent benignity. Diabetic angiopathy can develop within a few years of the onset of Cushing's syndrome.

Hypogonadism. Hypogonadism in Cushing's syndrome is primary and not due to gonado-

Table 14. Frequency of clinical manifestations in 450 cases of Cushing's syndrome. [According to SOFFER, DORFMAN, and GABRILOVE: The human adrenal gland (Philadelphia: Lea & Febiger 1961) and in 601 cases according to ROSS, MARSHALL-JONES, and FRIEDMAN: Quart. J. Med., N.S. **35**, 149 (1966)].

Clinical manifestation	Frequency (%)	
	According to SOFFER	According to ROSS
Moon face	88	75
Obesity	86	88
Hypertension	85	
Red face with "plethora"	77	
Amenorrhea in women	77	60
Hirsutism in women	73	65
Muscular weakness	67	61
Purple striae	60	
Hemorrhagic diathesis	59	42
Osteoporosis	58	
Ankle edema	57	
Buffalo hump	54	
Acne	54	45
Backache and other skeletal pains	54	40
Ecchymosis	52	
Mental changes	46	
Pathological fractures	38	
Impaired wound healing, leg ulcers	35	
Polyuria and nycturia	32	
Polydipsia	28	
Kyphosis	25	
Renal calculi	20	
Mild polycythemia	20	
Exophthalmus	14	

tropin deficiency, as was previously assumed. It must, therefore, be due to a direct inhibitory action of cortisol on the ovaries or testes. Amenorrhea often occurs, but is not an obligatory symptom. The corresponding pathologic anatomical finding is premature aging of the ovary. Pregnancy is possible, however, during untreated Cushing's syndrome. In the male, impotence is the predominant symptom. The testes may become smaller. No specific changes have been found in testicular biopsies.

The incidence of clinical signs and symptoms according to the statistics compiled by SOFFER (1961) and ROSS (1966) is shown in Table 14. The figures for individual series, such as those of PLOTZ (1952) and SOFFER (1961), and for our own 50 cases (FLURY, 1971) show only slight deviations.

NUGENT (1964) has proposed a formula based on the occurrence of clinical symptoms and simple laboratory findings for estimating the probability of the disease in patients with a confirmed or suspected diagnosis of Cushing's syndrome. He concludes that even without steroid estimations, the diagnosis can be made or excluded with great probability in half the cases.

Because of its therapeutic consequences, the diagnosis of Cushing's syndrome is of extreme importance and must be unequivocally founded on clinical experience as well as on reliable results of steroid determinations performed in a suitably equipped laboratory. As a rule, the patient has to stay in hospital for 8 to 14 days. A patient in whom Cushing's syndrome is only suspected, must never be referred to a surgeon for exploration of the adrenals. Cushing's syndrome may also be associated with adrenals of normal size, and the formation of scars after surgical inspection impairs or precludes subsequent adrenalectomy. In the fully developed syndrome, the clinical diagnosis is simple; but the patient should only be referred for adrenalectomy when the urinary levels of 17-hydroxycorticosteroids are found to be increased and are not suppressible by dexamethasone in a dose of 2 mg per day. In border-line cases, the decision as to whether Cushing's syndrome is actually present can be extremely difficult and sometimes impossible. In doubtful cases, it is best to wait several months and then repeat the laboratory tests.

γ) Laboratory Findings

ACTH. In patients with Cushing's syndrome of hypothalamic origin, the plasma ACTH levels, as measured by sensitive bioassays, are not usually found to be definitely elevated until the plasma corticosteroids have become normal following adrenalectomy and substitution therapy (NELSON, 1966). In the future, reliable radioimmunological methods of measuring ACTH may become of importance in the clinical diagnosis and especially in the differentiation of Cushing's syndrome of hypothalamic origin from cortisol-producing adrenocortical tumors and from paraneoplastic ACTH secretion.

Urinary Corticoids. Direct assessment of an increased cortisol secretion rate is of primary importance in the laboratory diagnosis of Cushing's syndrome. Urinary cortisol metabolites measured as 17-hydroxycorticoids (Porter-Silber chromogens) or 17-ketogenic steroids are usually found to be elevated (15 to 40 mg instead of 3 to 13 mg per 24 hours). Estimation of urinary corticoids alone does not always confirm or exclude a diagnosis of Cushing's syndrome. Values for urinary corticoids can be in the high normal range in confirmed cases of Cushing's syndrome or slightly elevated in obesity. Base-line excretion of urinary corticoids can only be evaluated in association with clinical findings and other laboratory tests.

Plasma Corticoids. Normal values of unconjugated plasma corticoids, as measured by the Porter-Silber reaction, vary between 6 and 25 μg per 100 ml between 7 and 9 a.m. and fall to 5 μg per 100 ml between 9 p.m. and midnight. The absence of a diurnal variation is suggestive of Cushing's syndrome, but not conclusive. It can also be due to brain tumors, cranial trauma, cardiac insufficiency, or psychosis. However, a normal diurnal variation is not consistent with the diagnosis of Cushing's syndrome.

Free Urinary Cortisol. Normally, less than 1% of the secreted cortisol is excreted unchanged in the urine, i.e. 20 to 150 μg per 24 hours. By contrast, values ranging from 200 μg to several mg per 24 hours are found in Cushing's syndrome.

17-Ketosteroids. The importance of urinary 17-ketosteroids in the diagnosis of Cushing's syndrome must not be overrated. They are elevated in about 50% of cases with adrenal hyperplasia. Strikingly elevated values, ranging between 30 and several hundred mg per 24 hours, are only found in adrenocortical carcinoma, and are generally associated with an increase in the 17-hydroxycorticoids. The 17-ketosteroids may, however, be reduced in adrenocortical adenomas, since the autonomous cortisol pro-

duction by the adenoma suppresses ACTH secretion, thus leading to atrophy of the normal adrenocortical tissue. High relative amounts of dehydroepiandrosterone among the 17-ketosteroids and of tetrahydro-S among the 17-hydroxycorticosteroids in the urine of patients with adrenocortical carcinoma are indicative of qualitative alterations of steroid biosynthesis in neoplastic tissue.

Cortisol Secretion Rate. Determination of the cortisol secretion rate by isotope dilution methods reveals increased 24-hour values, but is not necessary for diagnostic purposes.

Aldosterone, Pregnanediol and Pregnanetriol are not usually excreted in increased amounts. The urinary excretion of corticosterone metabolites and estrogens is occasionally elevated in hyperplasia and in carcinoma. For functional tests of the adrenal cortex, such as ACTH test, methopyrapone test, dexamethasone test, the reader is referred to pp. 385–390.

Plasma Electrolytes. Even in the absence of aldosterone excess, hypokalemia can develop due to the weak mineralocorticoid activity of cortisol, which is produced in excessive amounts. This is usually associated with metabolic alkalosis, with a pH of up to 7.5. The serum chloride concentration is usually low; the sodium level can be low, normal, or elevated. For a discussion of the pathogenesis of hypokalemia and alkalosis see p. 304. Severe hypokalemia is alway suggestive of paraneoplastic Chusing's syndrome. Calcium values are usually in the lower normal range and phosphate is normal or slightly reduced while phosphate excretion index (see Chap. XIV) is elevated. The alkaline phosphatase can be slightly elevated independently of osteoporosis. Hypercalcemia can occasionally occur after adrenalectomy. Thyroid function is normal; BMR and ^{131}I uptake can be decreased, elevated or normal. Serum cholesterol is usually slightly elevated.

Hematological Changes. Morphological changes in the blood are of some diagnostic value when complicated laboratory tests are not available. The eosinophilic leukocytes are most extensively influenced. They disappear entirely or can be present in only small numbers. The simple direct eosinophil count can be of diagnostic value. As a rule of thumb, Cushing's syndrome is unlikely to be present when the eosinophil count is more than 100 cells per mm^3 (normal values 100 to 300 per mm^3). Lymphopenia is almost always present in Cushing's syndrome. Moderate leukocytosis is less characteristic. The effects on the red blood picture are less impressive. Contrary to a prevalent opinion, polycythemia does not occur commonly in Cushing's syndrome. Hemoglobin values of 100 to 120% are often found, but the erythrocyte count is rarely elevated to the levels seen in polycythemia.

Carbohydrate Metabolism. Impaired carbohydrate metabolism can range from slightly reduced glucose tolerance to severe steroid diabetes. A normal fasting blood sugar does not by any means exclude Cushing's syndrome. The glucose tolerance test, however, almost always shows a rise of the blood sugar to values above 180 mg%, and a delayed return to normal. The intravenous insulin test usually reveals an inadequate fall

Table 15. Laboratory investigations when Cushing's syndrome is suspected

1. Single-dose dexamethasone suppression test (p. 389): Plasma corticosteroids at 8 a.m. before and at 8 a.m. after 1 mg of dexamethasone at midnight;
   when pathologic:
2. White and red blood status.
3. Direct eosinophilic count.
4. Serum chemistry: Sodium, potassium, chloride, urea, standard bicarbonate, fasting blood sugar, when normal glucose tolerance test.
   Urine: protein and sugar.
5. 17-ketosteroids, 17-hydroxycorticoids in 24-hour urine collection;
   when elevated:
6. 2 mg dexamethasone suppression test: 3 days 4 times 0.5 mg of dexamethasone, when 17-hydroxycorticoids > 4 mg – Cushing's syndrome.
7. 8 mg dexamethasone suppression test: 3 days 4 times 2 mg of dexamethasone,
   no suppression of corticosteroids: Cushing's syndrome due to tumor.
8. X-rays: skull lateral view, thoracic and lumbar spine lateral view, pelvis.
   Localization of suspected adrenocortical adenoma: retropneumoperitoneum with i.v. pyelography and tomography of the adrenals. Eventually selective phlebography and bilateral adrenal rein plasma cortisol.
9. Plasma corticoids at 8 a.m. and at 8 p. m. normal: 8 p.m. value 50% lower than 8 a.m. value and lower than 25 µg/100 ml; Cushing's syndrome: > than 25 µg/100 ml; both values identical. Perhaps free urinary cortisol (p. 382)

of blood sugar. The plasma concentrations of pyruvic acid and lactic acid are elevated (see p. 303).

δ) Radiology

The diagnosis of Cushing's syndrome is supported by radiography of the adrenal glands. Sometimes a straight abdominal X-ray alone can suggest an adrenal tumor, but diagnosis cannot be confirmed or be excluded by this means.

Only large adrenal tumors displacing the kidney are revealed by intravenous pyelography.

The retroperitoneal pneumogram, however, reveals three-quarters of all tumors, especially when combined with tomography. Differentiation between normal and hyperplastic adrenals is doubtful even with the best technique. The risk involved in the retroperitoneal pneumogram have been reduced to a minimum with the present-day method of using oxygen and presacral insufflation. Under local anesthesia, a pneumothorax needle is introduced to a depth of 3 to 5 cm at the midpoint between the anus and the tip of the coccyx, penetrating the ano-coccygeal ligament. One finger remains in the rectum during introduction of the needle. The simplest method of introducing the oxygen is to use a 50 cm^3 injection syringe and a three-way tap. After control aspiration 1200 to 1500 cm^3 of oxygen is introduced under mild pressure, the whole procedure being monitored by X-ray screening. Tomograms are made 1 to 2 hours later. Adrenal demonstration varies widely individually. Beautiful anatomical pictures of the adrenals can be obtained when the layer of adipose tissue between the kidneys and the adrenals is well developed.

Sometimes, however, the surrounding adipose tissue cannot be separated from the adrenal cortex in spite of a perfect technique, and evaluation of the adrenal size remains impossible. This occurs especially when fibrous changes are present in this area. Adrenal hyperplasia cannot be diagnosed with certainty by radiological means. Normal adrenal size is at the most 3–4 × 4 cm. Hyperplasia, however, can only be considered as being present when enlargement is pronounced and the effective radiographic magnification is taken into consideration. Normal adrenal size does not exclude Cushing's syndrome. Aortography is less suitable than renal angiography for demonstration of the adrenals. Occasionally, an adrenocortical tumor can be revealed by retrograde filling with contrast medium through a catheter introduced into the adrenal veins for bilateral blood sampling for cortisol determination (see p. 335 and Chap. VIII, p. 436).

An X-ray of the sella turcica is always indicated, although it is enlarged in only 10% of cases with Cushing's syndrome of hypothalamic origin. However, it is valuable to have a record of the original size of the sella turcica, because occasionally (6%) pituitary tumors develop after adrenalectomy. During childhood, a cortisol-secreting adrenal adenoma may lead to a small sella, which can become normal after treatment.

Lateral views of the thoracic and lumbar spine are always indicated for the detection of osteoporosis and assessment of its severity. Sometimes it is necessary to complement this with the a.-p. view of the pelvis and ribs, which may often reveal fractures with excessive callus formation.

Differentiation between adrenal hyperplasia and unilateral tumor can be achieved by scintillation scanning following administration of ^{131}I-19-iodocholesterol (BEIERWALTES, 1971).

ε) Special Forms of Cushing's Syndrome

1. Mixed forms of Cushing's syndrome and adrenogenital syndrome may be due to adrenal adenomas or carcinomas. They are rare in adults, but occur frequently during childhood. Hirsutism and acne of various degrees of severity are also found in pure Cushing's syndrome. Virilization, however, is always suggestive of an adenoma or of carcinoma. In these cases, the anabolic action of the androgens can compensate the catabolic effect of cortisol, thus preventing muscular atrophy. The output of abnormal steroid hormones by carcinomas of the adrenal cortex indicates deficiencies of individual enzymes. The majority of these abnormal steroids are precursors of normal hormones and their metabolites, such as compound S, tetrahydro-S, and dehydroepiandrosterone. According to the prevailing hormone, the features of Cushing's syndrome can be overshadowed by virilization, feminization, or hypertension and hypokalemia.

Finally, carcinoma of the adrenal cortex is often not accompanied by any endocrine symptoms, despite excessive steroid production, when most of the steroids are inactive precursors. These tumors can only be recognized when they are large and lead to pressure symptoms. Combined adrenocortical-medullary tumors are extremely rare. Pheochromocytoma may also lead to symptoms reminiscent of Cushing's syndrome, and cortical tumors to increased catecholamine production. Finally, Cushing's syndrome may be due to ectopic ACTH production by the adrenal medulla (MELONI, 1966).

2. Transitory and Periodic Cushing's Syndrome. A few cases of periodic cortisol excess and of spontaneous remission or healing of unequivocal Cushing's syndrome confirmed by hormone determinations have been described. This is extremely rare, so that postponement of treatment is never justified. In particular, conditions similar to Cushing's syndrome, which have been observed occasionally after cranial trauma, in encephalitis, and pulmonary tuberculosis, must not be mistaken for Cushing's syndrome, which can only be diagnosed by an increased secretion of 17-hydroxycorticoids not suppressible by 2 mg of dexamethasone. An "exogenous" Cushing's syndrome can only be induced by ACTH, cortisol or its derivatives.

3. Cushing's Syndrome due to Ectopic ACTH Production in Tumors (Paraneoplastic Cushing's Syndrome). Cortisol excess has been observed in hundreds of patients with non-endocrine tumors. It is characterized by the following features:

1. hypokalemia and alkalosis;
2. malignant course with an average life expectancy of 9 months;
3. clinical features of Cushing's syndrome may be absent;
4. marked pigmentation.

Small-cell carcinomas of the bronchus (50%), thymus (25%), and pancreas (10%) are the tumors most frequently involved, but Cushing's syndrome may also be due to other tumors of other organs, such as the gallbladder, parotids, colon, kidney, trachea, central nervous system, breast, prostate, and even other endocrine glands, such as the thyroid and adrenal medulla. Women are no more frequently affected than men. In about 50% of cases, the appearance typical of Cushing's syndrome and the typical symptoms are absent, although the cortisol secretion is definitely increased and the adrenals are always found to be hyperplastic. The absence of clinical symptoms may be explained by the rapid course. After surgical removal of the tumor, cortisol excess and its symptoms disappear; they reappear with the development of metastases. Hypokalemia and alkalosis, which indicate a considerable reduction of the amount of exchangeable potassium, are due either to the strikingly excessive cortisol production or to the secretion of corticosterone and compound S. CRANE (1966) found a 10-fold increase in the secretion rate of deoxycorticosterone in a case of Cushing's syndrome due to a bronchial carcinoma, but a normal rate in three cases of Cushing's syndrome of hypothalamic origin. The secretion rate of aldosterone is usually lowered. Diurnal variation of plasma cortisol is absent. The elevated excretion of urinary 17-hydroxycorticoids is not suppressed even by 8 mg of dexamethasone.

The pathogenesis of this syndrome has been explained by the demonstration of large amounts of a polypeptide with ACTH activity in the primary tumor, metastases, and blood, and a decreased ACTH content of the pituitary glands in these patients. In some of the tumors, more MSH than ACTH was found, which would explain the extreme pigmentation occasionally observed.

With the exception of the pigmentation, all signs and symptoms of Cushing's syndrome disappear after total adrenalectomy, but the deleterious course is due to the tumors, which are usually extremely malignant. Ectopic ACTH formation by a tumor should always be suspected when Cushing's syndrome is associated with pronounced hypokalemia; when it is found, the patients can be spared the useless adrenalectomy (see Chapter XVI, paraneoplastic syndrome).

4. Recurrent Cushing's Syndrome after Total Bilateral Adrenalectomy. Several well-documented cases of Cushing's syndrome recurring after total removal of both hyperplastic adrenals have been reported. This was probably due to local regeneration of residual adrenal tissue or to ectopic adrenocortical tissue becoming functional under increased ACTH secretion. Ectopic adrenocortical tissue has been found in different organs of the urogenital system, but also in the liver and pancreas. Only in the cases reported by CHAFFEE (1963) and NEY (1966) was ectopic adrenal tissue successfully localized at the lower or upper renal pole and surgically removed. Treatment with pituitary irradiation or methyrapone was attempted in all other cases. In one case described by STAUB (1967), multiple ectopic adrenal cortical regeneration occurred after complete pituitary destruction with radioactive yttrium, probably under the influence of ectopic ACTH produced by a pancreatic tumor.

5. *Primary Adrenocortical Adenomatosis (Nodular Dysplasia).* In a few cases of Cushing's syndrome in which the 17-hydroxycorticoid excretion was not suppressed by high doses of dexamethasone, surgery has revealed multiple nodules of the adrenal cortex and not the expected adrenal tumor. MEADOR (1967) found such alterations in a 14-year-old girl whose sella turcica was reduced in size and in whom no ACTH could be detected in the plasma after bilateral adrenalectomy even with the most sensitive assays. This indicated that the disease was due to a primary adrenocortical disorder.

6. Cushing's Syndrome in Childhood. In a few respects, Cushing's syndrome in childhood differs from Cushing's syndrome in the adult. In childhood, Cushing's syndrome is caused almost without exception by a benign or malignant adrenal tumor. Adrenal hyperplasia occurs in children very seldom. One of the most important features of Cushing's syndrome during childhood is growth retardation due to the antianabolic state of metabolism. This symptom is obligatory and any diagnosis of Cushing's syndrome in a child of normal size and with a regular growth curve is dubious. Osteoporosis can be very extensive. The intervertebral discs can become broader than the vertebral bodies. In the mixed forms, the anabolic action of androgens and the catabolic effect of glucocorticoids may counteract each other.

7. Cushing's Syndrome and Pregnancy. Pregnancy very rarely occurs in active Cushing's syndrome. Diagnosis may be difficult, because the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids and the plasma concentration of corticoids are elevated in normal pregnancy. In contrast to Cushing's syndrome, diurnal variation is maintained during normal pregnancy. Pregnancies in Cushing's syndrome often end in stillbirths. The fetal adrenals are small and the fetal zone is found to be prematurely regressed. There are many reports of adrenalectomy with continuation of the pregnancy in patients with Cushing's syndrome. An exacerbation of Cushing's syndrome due to an adrenal adenoma during pregnancy has also been observed (PARRA, 1966). Pregnancies are usually uncomplicated in women subjected to bilateral adrenalectomy for Cushing's syndrome and fully substituted.

f) Differential Diagnosis of Cushing's Syndrome

In the differential diagnosis of Cushing's syndrome, a combination of simple obesity, essential hypertension, and diabetes must first be considered; these constitutional diseases often occur together. The experienced clinician can recognize Cushing's syndrome from the specific facial expression. In contrast to constitutional anomalies, the case history indicates a definite time at which the illness started and character changes were noted. Differential diagnosis becomes more difficult when obesity is associated with red striae (which are not uncommon in rapidly developing juvenile obesity) and urinary 17-hydroxycorticoids are found to be elevated. Cortisol secretion rate is elevated in overweight subjects, but plasma corticoids and diurnal variation are normal. The 17-hydroxycorticoid excretion may be slightly increased. These patients may demonstrate certain constitutional characteristics (JACOBSON, 1964). After weight reduction, the cortisol secretion rate becomes normal in some patients, indicating that the increased cortisol turnover rate was due to the increased mass of adipose tissue with a higher capacity for lipid-soluble steroids. Striae in juvenile obese patients are said to be more pink than purple in colour, shorter, finer, and more numerous, and to be found not only on the lower abdomen and the hips, but also in the axilla, on the upper arms, the breast and the buttocks. However, this differentiation may be difficult in individual cases. The pathogenesis of red striae is explained in both instances by stretching of the skin, increased subcutaneous adipose tissue, and simultaneous involution of the elastic fibers due to the increased amount of cortisol. As a rule, obesity with striae can be differentiated from true Cushing's syndrome by the following laboratory tests:

1. the increased excretion of urinary 17-hydroxycorticosteroids is suppressible by dexamethasone in a dose of 2 mg per day (see p. 389) in obesity but not in Cushing's syndrome;
2. in obesity, there is a circadian rhythm of plasma corticosteroids, in Cushing's syndrome, there is a plateau;
3. excretion of free urinary cortisol is very low in obese as well as in healthy subjects, but is elevated in Cushing's syndrome.

The "diabète des femmes à barbe" of ACHARD and THIERES is, in our opinion, not a clinical entity, but a random coincidence of simple hirsutism and vegetative glycosuria caused by an accident in a 69-year-old woman. This suggestive term has since been used to describe several cases of Cushing's syndrome.

Virilization with normal or only insignificantly elevated urinary 17-ketosteroids is suggestive of an ovarian tumor (see p. 634).

The differentiation of Cushing's syndrome from Stein-Leventhal syndrome (see p. 609) can be difficult. The latter diagnosis can only be confirmed by the finding of specific ovarian alterations, whereas Cushing's syndrome is diagnosed from the increased corticoid excretion and the metabolic disorders.

For the differentiation from adrenogenital syndrome compare p. 358.

g) Course, Prognosis and Cause of Death

The course of untreated Cushing's syndrome is deleterious, although a few cases of spontaneous remission have been described (see p. 353). This occurs too rarely to justify any delay in

therapy. The course of the disease is extremely variable. The illness can lead from full health to death within 6 months or it can last for many years. There are known cases in which the illness lasted for over 20 years; this was, however, at least partly due to remissions induced by pituitary X-ray irradiation. In general, a patient with untreated Cushing's syndrome has a life expectancy of 3 to 10 years, on average of 5 years. The major cause of death is now hypertension and its complications. Before the era of antibiotics, death was due predominantly to infections. Resistance to infection is reduced in Cushing's syndrome, and staphylococcal and streptococcal infections are especially common; the incidence of fatal tuberculosis, however, is not higher than in the normal population, but tuberculosis does tend to have a malignant course in preexisting Cushing's syndrome. The third major cause of death is carcinoma. The earlier assumption of any increased predisposition to carcinoma has now been explained by the relative frequency of paraneoplastic Cushing's syndrome. In this condition, the life expectancy ranges from 30 days to 2 years with an average of 9 months. Complete cure through removal of the primary tumor has only been described once. The prognosis of adrenocortical carcinoma is poor. Only half the cases survive for more than 2 years and only an isolated few survive for over 5 years in spite of o,p'-DDD therapy.

h) Therapy

When an adrenal tumor has been diagnosed from X-rays or an adrenocortical function test with corticosteroid determinations, the only possible course of action is surgery. When preoperative findings favor a diagnosis of bilateral adrenal hyperplasia, there are three possible therapeutic approaches:

1. medical treatment with anabolic hormones or adrenostatic drugs;
2. pituitary irradiation;
3. surgery: adrenalectomy, hypophysectomy.

α) Anabolic Hormones

Testosterone or other anabolic steroids, which – at least in theory – can normalize the negative nitrogen balance, have at the most a palliative effect and can only be used for preoperative preparation. Estrogens, which should theoretically act by promoting cortisol binding to transcortin, are not sufficiently active to influence the course of the illness.

β) Adrenostatic Drugs

Amphenone-B can totally block adrenal cortical hormone production, but cannot be administered for long periods because of its toxicity.

Methopyrapone, an inhibitor of 11β-hydroxylation, inhibits the production of cortisol, but leads to excessive production of compound S and deoxycorticosterone, resulting in hypertension or edema.

o,p'-DDD (2,2-[4-chlorophenyl, 2-chlorophenyl] 1,1-dichloroethane) is beneficial for patients with inoperable adrenocortical carcinoma, although it is not free of toxic side effects such as anorexia, diarrhea, vomiting, and depression. Before administration of o,p'-DDD, as much as possible of the tumor tissue should be surgically removed. When a radical operation is not possible and the urinary excretion of 17-ketosteroids and 17-hydroxysteroids remains elevated or increases again with the formation of metastases, o,p'-DDD must be given continuously or intermittently in a daily dose of up to 10 mg. It has been ineffective in 20 to 25% of cases so far, but in two-thirds of the cases regression of the hormone production by the adrenal cortex has been achieved, associated with disappearance of the symptoms of Cushing's syndrome; in some cases substitution therapy has even become necessary. In one third of cases, mainly juvenile patients, primary tumor and occasionally even the metastases completely regress. Some patients have been successfully treated for up to two years with this drug. A few became refractory; permanent recovery has not yet been observed. Formation of metastases was not avoided by prophylactic postoperative treatment. The production of the biologically active corticosteroids and androgens is suppressed within the adrenals by inhibition of several enzymes, mainly of 3β-hydroxydehydrogenase, but the drug also affects the peripheral metabolism of steroid hormones.

Trial therapy with triparanol, an inhibitor of cholesterol biosynthesis which is not completely free of side effects, has been only partially successful.

Aminoglutethimide. This substance was developed as an antiepileptic drug under the trade name of Elipten. Animal experiments have shown that it inhibits the conversion of cholesterol to pregnenolone, and thus the biosynthesis of cortisol and corticosterone, by blocking adrenal desmolase (DEXTER, 1967). Results of the first clinical trials indicate that aminoglutethimide is suitable for the suppression of excessive steroid production by autonomous

adrenal tumors (Cushing's syndrome due to adrenal adenoma or carcinoma, primary aldosteronism), whereas the drug diminishes cortisol secretion rate only slightly in patients with Cushing's syndrome with bilateral adrenal hyperplasia. In healthy subjects, it does not induce a decrease in cortisol secretion rate, because its action is compensated by an increased ACTH production, but it does cause a decrease in aldosterone secretion rate (FISHMAN, 1967). Suppression of thyroid function (see p. 193) has been described and has to be treated by substitution therapy. Side effects such as allergic skin reactions or nausea are relatively frequent (HORKY, 1968).

The search for new, more effective and less toxic adrenostatic drugs, however, appears very promising.

γ) Radiotherapy

X-ray irradiation of the adrenals has been ineffective, whereas intensive radiation of the pituitary gland has been really successful in a few cases. The chance of success with conventional X-ray radiation is estimated at 10 to 25%; recurrences are frequent. The X-ray dose is limited to 3000 r because of the risk of damage to the surrounding organs. The application of strongly accelerated alpha particles or protons, which is possible in centers with special accelerators, appears to be considerably more promising according to the first reports of success. The incidence of recurrences with this new radiation method is not yet known. The success of pituitary irradiation can only be assessed after a waiting period of 6 months.

Although pituitary irradiation has been almost completely abandoned because total adrenalectomy is more reliable, it is gaining renewed importance in view of the occurrence of pituitary adenomas in a certain percentage of patients with Cushing's syndrome who have been treated by total adrenalectomy.

Pituitary irradiation may be tried first in mild cases of Cushing's syndrome with a chronic course and when a pituitary tumor has been clinically or radiologically detected. However, when the course is severe and progressive, no time must be lost and total adrenalectomy is the treatment of choice.

Transsphenoidal implantation of ^{90}Yt or ^{198}Au, possibly combined with ^{192}Ir, wich has recently been improved by a stereotactical method (MUNDINGER, 1967) destroys 70 to 90% or 30 to 50% of the pituitary gland respectively, and may cure Cushing's syndrome. On the positive side, it does not involve severe stress to the patient, but dosage is difficult, loss of the other pituitary hormones is possible, and the surrounding organs may be damaged, in particular the optic nerve with loss of visual fields, paralysis of the ocular muscles, and injury to the blood vessels. When the more effective ^{90}Yt is used, cerebrospinal fluid fistulas occur sooner or later in 20 to 30% of the cases. Better results are obtained when the puncture opening is closed with a screw. Isotope implantation is beginning to be replaced by stereotactic high-frequency electrocoagulation of the pituitary gland, which is associated with less complications (ZERRAS, 1965) or by the microsurgical transnasal-transsphenoidal operation according to HARDY (see Chap. V, p. 115). For the time being, isotope implantation appears to be justified only where adrenalectomy and hypophysectomy are contraindicated.

δ) Operations

1. Adrenalectomy. Total bilateral adrenalectomy is still the only reliable way of treating Cushing's syndrome with bilateral adrenal hyperplasia, and is thus the therapy of choice. Since it has become known that a pituitary tumor can develop and that the growth of a preexisting pituitary tumor can be accelerated after adrenalectomy, misgivings have come up about this form of treatment. After removal of the hyperplastic adrenals, plasma ACTH rises to high levels, and apparently there is a positive correlation between this increase and the probability of a pituitary tumor developing later on (NELSON, 1966). Nevertheless, we still consider total adrenalectomy the treatment with the best chance of success and with least risks. Up to 1963, 28 reactive pituitary tumors growing after adrenalectomy had been reported. Twelve of these were already known to be present before surgery (KRACHT, 1963). It is difficult to assess the incidence of these reactive pituitary tumors among all cases of total adrenalectomy, because this operation is often performed and not always reported in the literature. In a series reported by the Mayo Clinic, the incidence of reactive pituitary tumors growing after adrenalectomy was found to be 7%. Growth of these pituitary tumors proceeds slowly and can be detected at an early stage if frequent check-ups are performed. Treatment is necessary in only about half the cases. Radiotherapy is the treatment of choice.

When total adrenalectomy is performed, the treatment of Cushing's syndrome is purchased at the price of Addison's disease, and the patient is then dependent on substitution therapy for the rest of his life. In spite of these disadvan-

tages, i.e. the small risk of a reactive pituitary tumor and the necessity of substitution therapy, we still consider total adrenalectomy as the only reliable way currently available of converting a life not worth living and with an average life expectancy of 5 years into a symptom-free life with full activity and practically normal life expectancy. The development of more effective and harmless adrenostatic drugs or radiotherapeutic methods may alter the situation in the future.

Subtotal adrenalectomy should no longer be performed; it is only effective when 90 to 95% of the adrenal tissue is resected, which necessitates a substitution therapy in any case in almost half the patients, and the risk of recurrence is very high (25%). Since the realization of how high the plasma ACTH concentration can rise after adrenalectomy in patients with Cushing's syndrome, it has become apparent that it is only a matter of time before the residual tissue regenerates and resumes production of excessive amounts of cortisol. Finally, the risk of acute adrenocortical insufficiency is considerably higher after subtotal adrenalectomy without substitution therapy than in substituted and well-instructed Addisonian patients. The residual tissue is under maximum ACTH stimulation and cannot fulfill increased demands due to additional stress. The risk of the development of a reactive pituitary tumor is presumably the same after an effective subtotal adrenalectomy and after total adrenalectomy with substitution therapy.

At present, the following procedure is recommended: when the sella is normal, total adrenalectomy is performed. In mild cases only, a trial with radiotherapy may be considered. It must be remembered that a preoperative diagnosis of hyperplasia is never unequivocal and that an adenoma or carcinoma can be definitely excluded only by surgical revision. When the sella is enlarged, irradiation of the pituitary gland is recommended, before or after adrenalectomy. Again, only in the mild cases is there time to wait for the effects of irradiation. In severe cases of Cushing's syndrome, total adrenalectomy must immediately follow irradiation. We consider a neurosurgical or transethmoidal resection only when a pituitary tumor causes neurological complications, in particular a loss of visual fields.

Removal of both adrenals at the same operation is preferable to the previous two-stage method. It is unrealistic to expect a remission after the removal of only one adrenal. It occurs only in very rare instances. The disadvantage of the greater surgical stress is outweighed by the considerable advantage of terminating the illness at once instead of over weeks or months, and of avoiding a repetition of general anesthesia and surgical trauma.

Adrenalectomy has to be carried out in a medical center where a team of experienced surgeons, clinical endocrinologists and anesthesiologists is available. The operation is complicated technically by the obesity of the patients, their low resistance and their increased bleeding tendency. Small residues of adrenal tissue lead to recurrence, so that success is merely a temporary illusion; a second operation is generally impeded by the formation of scar tissue. Medical pre- and postoperative treatment and supervision are essential to the success of the operation. The mortality is less than 5%.

At present, the transdiaphragmatic, extrapleural approach is usually chosen for adrenalectomy, necessitating a bilateral incision along the 11th rib. In thin patients only, a transabdominal approach with a wide incision for both adrenals is feasible.

The following procedure is recommended during operation: if exploration on the right reveals an atrophic adrenal, in all probability there is a tumor on the left side. The atrophic adrenal is left intact and the tumor on the other side is removed. When a tumor is found on the first side, it is removed and the other side is left intact. However, when the right adrenal is found to be normal or hyperplastic, it is totally removed. The left adrenal is then also removed. See Table 8 for substitution during and after operation.

2. Hypophysectomy. In theory, hypophysectomy may also be considered in Cushing's syndrome with adrenal hyperplasia. Several approaches are possible: the neurosurgical transfrontal or better paranasal transethmoidosphenoidal, the microsurgical transnasal-transsphenoidal method (HARDY, 1969), stereotactic implantation of β- and γ-emittors (MUNDINGER, 1967) or stereotactic electrocoagulation (ZERRAS, 1965) (see p. 115). Advantages are a lesser surgical stress and the removal or prophylaxis of a pituitary tumor, but these are offset by the disadvantages of possible thyroid or gonadal failure and of the development of mild diabetes insipidus. Radical hypophysectomy is difficult, and after partial removal there is a tendency for regeneration and recurrence, particularly in Cushing's syndrome, in which the pituitary is under neurohumoral stimulation by the hypothalamus. Moreover, adrenocortical tumors cannot be excluded with absolute certainty, even with the most perfect diagnostic technique, except by surgical revision. We therefore recommend hypophysectomy only when the patient's con-

dition precludes adrenalectomy, when reactive pituitary tumors grow to dangerous proportions in spite of irradiation, or in the extremely rare cases of primary enlargement of the sella, particularly when associated with loss of visual fields.

3. Postoperative Course. The operation and postoperative course can be well controlled, initially by intravenous, later by oral administration of cortisone. However, the cortisone dosage must not be reduced too quickly. Rapid reconstruction of tissues may lead to the post-adrenalectomy syndrome, which is characterized by restlessness, tachycardia, and other vegetative circulatory disorders, anorexia, nausea, vomiting, and abdominal pains. In most cases, these symptoms can be corrected by temporarily increasing the dose of cortisone. Occasionally, there is a fall in blood pressure due to hypovolemia or tachycardia with pale, cold, moist extremities and nausea necessitating treatment with infusions of plasma or dextrane mixtures. Occasionally, often only after a few months, subacute hemorrhagic pancreatitis of unknown pathogenesis may develop. There may be hypercalcemia in the presence of normal serum phosphate levels. When these signs are observed, the patient must immediately be treated again with cortisone, with a daily dose up to 200 mg. The patients go through a critical phase between the 10th and the 20th postoperative days. They must definitely be kept under hospital supervision for at least that period. When, in cases of adrenal tumors, atrophy of the contralateral adrenal is suspected, synthetic long-acting ACTH, 1 mg twice daily, can be given i.m. for a few days even before the operation. Postoperatively, the same dose is continued for about a week; thereafter, it can be reduced to 1 mg once daily for another week. Sometimes, however, the atrophic adrenal recovers only after some months or not at all. In a few cases, persistent failure of ACTH secretion has been observed, lasting for years and necessitating continuous substitution therapy with cortisone.

The first signs of success appear within a few weeks, as soon as the daily dose of cortisone is reduced to 50 mg. The premorbid condition is not generally recovered for 3 to 6 months, and the patients must be well supervised during this period. The blood pressure falls to the original values, unless it is fixed by irreversible vascular changes. The diabetes almost always disappears. Excessive fat deposits are broken down, and the pendulous folds of abdominal fat must sometimes be corrected by plastic surgery. The skin usually peels after 6 to 8 weeks, as after scarlet fever. There is often loss of hair, which is immediately replaced by new hair, occasionally different in quality and color. Pigmentation of the skin can be of varying severity. Menstruation recommences after 2 to 3 months, and the chances of pregnancy after successful treatment of Cushing's syndrome are good. Mental changes usually regress after successful treatment. Individual symptoms, such as loss of libido, may persist. In the woman, this can be treated with small doses of androgens.

4. The Adrenogenital Syndrome

A. PRADER and M. ZACHMANN

The term adrenogenital syndrome describes the clinical states induced by overproduction of

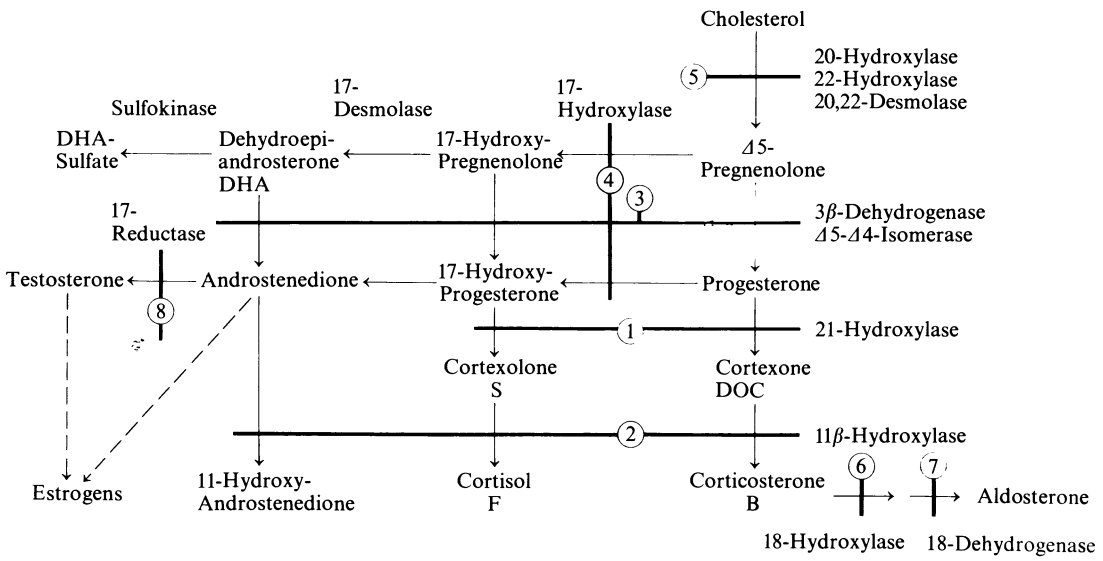


Fig. 27. Known hereditary enzyme deficiencies in the biosynthesis of adrenocortical and gonadal steroids

Table 16. Hereditary disturbances of steroid biosynthesis (enzyme deficiencies and clinical pictures)

Enzyme deficiency	Gonads	Adrenal cortex				Clinical picture
	Intersexual genitalia in boys	Intersexual genitalia in girls	Progressive virilization	Salt loss	Hypertension	
With adrenal hyperplasia						
1. 21-Hydroxylase without salt loss	−	+	+	−	−	Congenital adrenogenital syndrome
with salt loss	−	+	+	+	−	
2. 11β-Hydroxylase	−	+	+	−	+	
3. 3β-Dehydrogenase	+	(±)	(±)	+	−	
4. 17-Hydroxylase	−/?	−	−	−	+	Irregular syndrome (p. 336)
5. 20,22-desmolase						Lipoid hyperplasia of the adrenals (p. 323)
Without adrenal hyperplasia						
6. 18-Hydroxylase	−	−	−	+	−	Hypoaldosteronism (p. 325)
7. 18-Dehydrogenase						
8. 17-Reductase	+	−	−	−	−	Atypical (partial) testicular feminization (p. 725)
9. 17,20-Desmolase	+	−	−	−	−	

androgenic steroids by the adrenal cortex. This group is well characterized clinically. In the last few years, increasing knowledge about the mechanisms involved in steroid synthesis has led to the discovery of clinical conditions which are closely connected with the congenital forms (see below) of the adrenogenital syndrome biochemically but are clinically quite different. They are not associated with overproduction of androgens and only some are associated with hyperplasia of the adrenal cortex. Fig. 27 and Table 16 give a survey of the congenital disorders of steroid biosynthesis currently known. Only the various forms of the true adrenogenital syndrome are discussed in this section. Since the other disorders in the biosynthesis of adrenocortical steroids become clinically manifest in different ways, they are dealt with in the appropriate chapters (lipoid hyperplasia, p. 323; 17-hydroxylase defect, p. 371; 17,20-desmolase deficiency, p. 726; 17-reductase deficiency, p. 726; hypoaldosteronism, p. 325).

a) Classification, Definition

The adrenogenital syndrome does not always have the same etiology and pathogenesis. Overproduction of androgenic steroids of the adrenal cortex is, however, common to all forms. Three main forms are differentiated on the basis of pathogenetic and therapeutic features: 1. the hereditary adrenogenital syndrome due to congenital adrenocortical hyperplasia (see below for the different forms), 2. the adrenogenital syndrome with acquired adrenocortical hyperplasia, and 3. the adrenogenital syndrome resulting from an adrenocortical tumor.

The symptomatology of the overproduction of androgenic steroids is, of course, much more striking in the female than in the male, regardless of whether the androgens arise from the adrenal cortex or from the gonads. The symptomatology is referred to as virilization in the female sex, whereas in the male sex no special term is available as there is no characteristic set of symptoms. Unfortunately, the terms virilization, hirsutism, and hypertrichosis are generally used rather inaccurately. This results in obscurity and confusion, so that definition of these terms seems indicated before discussion of the individual syndromes. The term *virilization* is used in girls or women when 1. the secondary sexual characteristics are predominantly masculine, 2. female secondary sexual characteristics are absent or are undergoing regression, and 3. the clitoris is hypertrophic. This triad of symptoms only occurs during treatment with high doses of testosterone or in association with an increased androgen production due to either an adrenogenital syndrome or an androgen-producing tumor.

Hirsutism signifies a male type of hair distribution in women – increased sexual, body, and facial hair. It is sometimes accompanied by acne without the development of other androgenic symptoms such as hypertrophy of the clitoris or deep masculine voice. Hirsutism is usually idiopathic (p. 377), but may arise with ovarian disorders (Stein-Leventhal syn-

drome, p. 593) or adrenal disorders (Cushing's syndrome, p. 345).

Hypertrichosis implies increased body hair without accentuation of the sexual hair. It can, therefore, arise in children before development of the secondary sexual characteristics and has nothing to do with androgens.

b) Hereditary Congenital Adrenogenital Syndrome (Congenital Virilizing Hyperplasia of the Adrenal Cortex)

These conditions are classic examples of "inborn errors of metabolism" due to enzyme defects. The large number of specific enzymes required for the synthesis of cortisol from cholesterol (see p. 358, Fig. 27) explains the multiplicity of the clinical symptoms. There is one factor common to all enzyme defects so far known and associated with adrenocortical hyperplasia: cortisol synthesis or rate of production is diminished, and there is an accumulation of intermediary metabolic products due to the absence or reduced activity of enzymes required for their further processing. Depending on type, amount, and biological activity of these intermediate metabolic products, clinical features such as virilization, hypertension, salt loss, and (due to a decrease in cortisol) even Addisonian crises, poor resistance and hypoglycemia can arise.

α) Frequency

Statistics on the frequency vary from about 1 : 500 (Eskimos) to 1 : 67000. In Switzerland, the frequency is found to be 1 : 5000 (PRADER, 1962). It is approximately the same in both sexes. In the past it was assumed that the frequency was higher in the female sex, but this can be explained by the more difficult diagnosis in male patients and the fact that male patients with milder forms do not consult their doctors.

β) Heredity

The condition very frequently occurs in siblings. Consanguinity between parents is uncommon or lies many generations back and is thus insignificant. Statistical investigations indicate an autosomal recessive inheritance. Affected siblings also demonstrate the same enzyme defect, but not always to the same extent (ROSENBLOOM, 1966). Heterozygotes are common. If the rather low frequency of 1 : 40000 for homozygotes is accepted, at least 1% of the population must be heterozygous. Other authors accept a gene frequency of 1 : 16 (ROSENBLOOM, 1966) or 1 : 35 (PRADER, 1962). The ability to recognize heterozygotes would have a decisive effect on genetic counselling, but no satisfactory method has yet been found. Pregnanetriol excretion increases more in response to ACTH in heterozygotes for the 21-hydroxylase defect than in normal controls (see below), but this is not a safe way of identifying individual heterozygotes. As in the healthy, no pregnanetriolone is found in the urine of heterozygotes (see below). It may be possible later to detect heterozygotes for the 21-hydroxylase defect by estimation of 17α-hydroxyprogesterone or testosterone in the plasma after stimulation with ACTH.

γ) Pathogenesis

Theories on the pathogenesis of congenital hyperplasia of the adrenal cortex have changed with development of specific methods of steroid analysis. Originally, overproduction of androgens was claimed to be the primary pathogenic factor. It only became obvious after the introduction of specific methods that not only are certain steroids present in increased amounts but the production of other steroids is reduced. The idea that the reduced production of cortisol must be considered as the primary pathogenic factor became prevalent. Furthermore, it has also been shown that the cause of the diminished cortisol production is not the same in all cases but that synthesis is interrupted at different stages (Fig. 27). Deficiency of 3β-dehydrogenase, 21-hydroxylase and 11β-hydroxylase can cause a reduction in cortisol production and an adrenogenital syndrome.

Since cortisol is the chief regulator of ACTH secretion, a decrease in the amount of circulating cortisol can lead to the release of CRH (corticotropin releasing hormone) from the hypothalamus and thus to the liberation of increased amounts of ACTH from the anterior pituitary. The concentration of ACTH demonstrated by radioimmunological methods is thus found to be elevated in all forms of congenital hyperplasia of the adrenal cortex. The increase in ACTH secretion results in increased production of only those intermediary steroids formed before the particular enzyme block. The block, however, causes little or no increase in cortisol production, and ACTH secretion is not inhibited. Thus, hyperplasia of the adrenal cortex can be interpreted as a result of continuous overproduction of ACTH. The reason for the increased skin pigmentation found in some cases is the same as for that in ADDISON'S disease (see p. 317).

The enzyme deficiency is not absolute in the majority of cases of congenital hyperplasia of the adrenal cortex, i.e. the minimum vital

amount of cortisol is practically always produced. The degree of the cortisol deficit varies from case to case, and with a few exceptions (hypoglycemia, poor resistance) is not sufficient to become clinically obvious. In contrast, the deficient response of glucocorticoid production to ACTH is definitely perceptible. Steroid anomalies associated with adrenocortical hyperplasia have two common characteristics – they are accentuated by ACTH and normalized by glucocorticoids which inhibit ACTH.

δ) Pathologic Anatomy

R. E. Siebenmann

The reader is referred to the description of the clinical features (p. 364) for a discussion of the effects of androgen excess on the genital organs and on the organism as a whole. The effects of salt loss and hypertension in the special forms of the virilizing syndrome are described in the same section. The only other important pathologic changes are those found in the adrenals, the gonads and the pituitary gland (Dhom, 1965; Symington, 1969; Siebenmann, 1973).

The only observations so far recorded on the *uncomplicated, simple virilizing syndrome* (21-hydroxylase deficiency without salt-losing syndrome) are autopsy and biopsy findings in children and adults. A latent terminally decompensated salt-losing syndrome cannot always be excluded in children dying from the condition.

A fairly characteristic bilateral *hyperplasia of the adrenal cortex* is found in all confirmed cases (Fig. 28). In children the glands may be 4–10 times larger than normal, and in adults 6–8 times larger. The increase in cortical mass parallels to a certain degree the severity of the genital masculinization and the virilization. The cortical hyperplasia is initially diffuse, but always becomes irregularly nodular with advancing age. The surface, slightly folded at first, becomes increasingly knotted. Even in children the cut surface is intense brown in color, turning to dark brown with advancing age. Narrow zones of yellowish cortex may be found only in the subcapsular peripheral or in the central cortical area.

The histological structure of the cortex can only be studied as long as the hyperplasia is diffuse and not distorted by nodular growth, i.e. during childhood. The *zona glomerulosa* is then found to be definitely wider than normal. This layer is followed by a narrow *zona fasciculata*, built up of lipid-containing spongiocytes. In autopsy cases this zone is usually also lipid-depleted. The thickness of the zone varies and there is no definite border between it and the inner *zona fasciculo-reticularis*, which forms the main mass of the cortex. It consists of lipid-free "compact" cells. They are definitely enlarged and exhibit all signs of intensive activity. Intensive activity of phosphatases, succinic dehydrogenase and diaphorase can be demonstrated by enzyme-histochemical means. The activity of 3-β-hydroxysteroid-dehydrogenase, the only histochemically demonstrable enzyme of the biosynthetic steroid pathway, is marked. The 21-hydroxylase-deficiency has been demonstrated in incubation studies on slices of such glands, but cannot be shown by enzyme-histochemical means. In addition, the cells of this hyperplastic layer are loaded with an extraordinary amount of *lipofuscin* responsible for the deep brown color of the cortex. The pigment does not differ from that normally found in the adrenal cortex, but the reason for its excessive accumulation is not known. The outer layer of this zone still shows a fascicular structure, while further in toward the medulla the structure is predominantly reticular. It is this zone which forms more and more nodular and even adenomatous masses with advancing age. They extend right up to the capsule, often perforating it in a hernia-like manner, or break up the medullary tissue internally. Separated, distorted or flattened remnants of fasciculata or glomerulosa are always found. Whereas encapsulated and probably autonomous adenomas have frequently been observed anatomically, only two cases of cortical *carcinoma* have been described in this type of cortical hyperplasia.

Not only the normally situated tissue is found to be hyperplastic, but also *ectopic adrenocortical tissue*. Thus, in the female uni- or bilateral cortical nodules, which are sometimes tumorous, arise in the mesovarium and broad ligament. In the male, nodular proliferations occur in the epididymis and testis and may almost completely replace testicular tissue, although they probably always arise from the aberrant cortical tissue which commonly occurs in the hilar region of the testis. The nature of these testicular nodules, however, is still a matter of debate, since they cannot be distinguished from interstitial cell tumors by either morphologic or biochemical means. On the other hand, the question arises as to whether the cases of bilateral interstitial cell tumors are not in fact cases of unrecognized congenital adrenogenital syndrome. Regression in size of such testicular masses during cortisone treatment suggests that they are of adrenocortical origin.

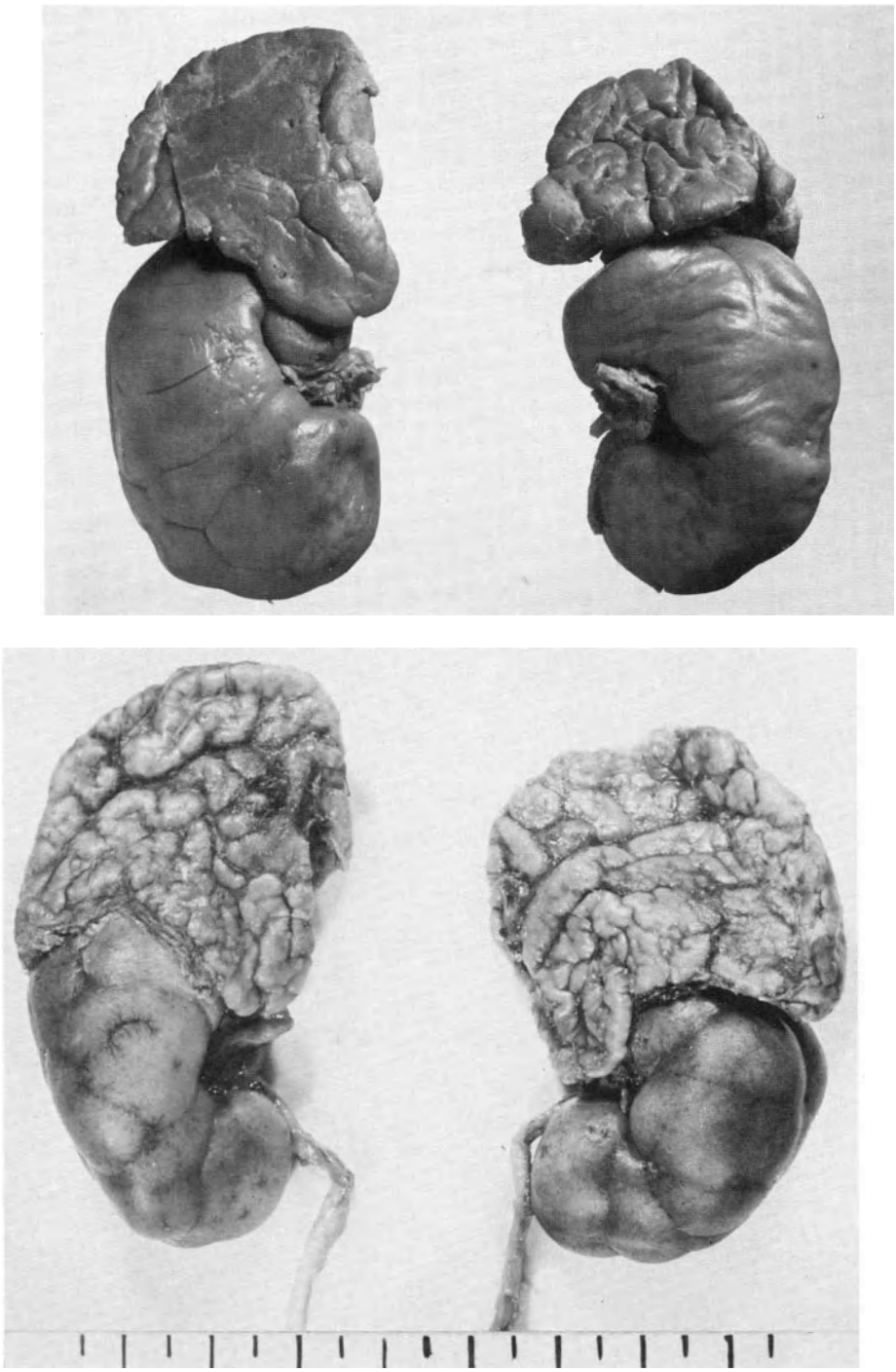


Fig. 28. Above: bilateral diffuse adrenocortical hyperplasia with moderate folding and segmentation of the surface in a 5-year-old girl with congenital adrenogenital syndrome (MB 2302/63). Below: cerebriform adrenocortical hyperplasia in an 18-day-old infant with congenital adrenogenital salt losing syndrome (SN 969/55)

The *testes* are definitely abnormal in all severer forms. During childhood there is some acceleration of tubular maturation up to the spermatocyte I, but the glands remain small, thus contrasting with the true precocious puberty, especially as no Leydig cells are found. They remain small also in the adult. The tubules show some incomplete spermatogenesis up to the spermatocyte I, fibrosis develops and no Leydig cells are found. Milder forms of the

syndrome may be responsible for some of the cases of spermatogenic arrest without further endocrine disorders. Exceptionally, intact spermatogenesis and fertility are observed even in untreated males.

The *ovaries* are usually normal in girls, but pubertal growth and maturation fail to occur in the fullblown syndrome, follicular development does not continue beyond the antrum stage, and ovulation and corpus luteum formation do not occur. The number of primordial and primary follicles diminishes rapidly, so that at the age of 30 years no more are found. In adults the number of usually small, but sometimes even large follicular cysts is increased and the result is the "*small sclerocystic ovary*". In contrast to the Stein-Leventhal ovary there is no stromal hyperplasia and no hyperthecosis. The ovaries have not been studied in milder cases with aberration of ovarian function only.

The *pituitary* does not show characteristic changes in the anterior lobe. An increase of the sparsely granulated mucoid cells cannot be distinguished from that observed in any stress situation. No Crooke cells are found.

In the *salt-losing form* of 21-hydroxylase deficiency the endocrine organs show quite a different morphology. The *adrenals* of these infants are huge, their relative weight being increased to 6 to 10 times above the normal. Their surface is very characteristically folded and reminiscent of a cerebral cortex, so that the term "cerebriform hyperplasia" has been coined (Fig. 28).

The cortex is several times thicker than normal even in an infant of 2–4 weeks, and is thrown up in high folds. They often form secondary folds, become superimposed and tightly packed together. The bulk of this cortex shows a *fascicular* structure. There is no zona glomerulosa and only the innermost layers, at the junction with the degenerating remnants of fetal cortex, exhibit some reticular arrangement of their cells. As a rule, all cortical cells are enlarged as well as their nuclei and mitoses are often found in the outer cortical layer. The cytoplasm is usually quite devoid of lipids, and granular; it contains vacuoles of hydropic degeneration, but never any lipofuscin. Accordingly this cortical tissue is of dirty gray color. The typical folding is the result of two parallel processes: The inner, fetal cortex, which is still present a few days after birth in the youngest patients examined, undergoes complete involution as in the normal subject, although this process is somewhat delayed. It is the definite "adult" outer cortex which builds up the typical folded cortex by intensive hyperplasia after birth. From observations in very young infants it can be assumed that the fetal cortex was definitely hyperplastic before birth. Accessory adrenocortical tissue shows the same type of hyperplasia. It is therefore more easily detected at the usual sites.

The *gonads* are found to be normal in both sexes. The *pituitary*, in contrast, always shows marked changes of the anterior lobe cells. The total number of sparsely and fully granulated mucoid cells and of large chromophobes is significantly increased at the expense of the acidophils. In addition to the cellular pattern consistent with an increased production and secretion of ACTH in cortisol deficiency, the fully granulated mucoid cells – formerly the basophils – are also increased for some unknown reason. This increase, however, is also a reaction to the adrenocortical disorder, and is reversible by corticosteroid treatment.

In summary the pathologic findings in both these forms of 21-hydroxylase deficiency are consistent with the present theory on their pathogenesis. They suggest a primary disorder of adrenocortical secretion. In the *uncomplicated* form a not very marked, but constant increase in ACTH stimulation causes not only lipid depletion and broadening of the inner fasciculo-reticular layer of "compact cells", but also a massive proliferation of this "functional" layer until a quantity of cortisol compatible with life is produced by these deficient cells. The hypertrophy of the zona glomerulosa reflects the secondary hyperaldosteronism compensating the latent salt loss in these patients, as is borne out by the hypertrophy of the *juxtaglomerular* apparatus found in these cases. In the *salt-losing form* the cortex is found to be under maximal stimulation, in keeping with the more severe cortisol deficiency, and the anterior pituitary reflects this situation. The absence of a zona glomerulosa corresponds to the equally deficient production of aldosterone. Juxtaglomerular cells are also increased in number, although to a lesser degree.

Biopsy and autopsy in cases of the rare *11-β-hydroxylase-deficiency* have so far revealed the same hyperplasia of the fasciculo-reticularis as is seen in the simple virilizing form. In addition, hypertrophy of the zona glomerulosa has been found, which seems to be integral to the disorder of cortical secretion and not due to secondary hyperaldosteronism. There is usually no salt loss in these patients and no hypertrophy of the juxtaglomerular apparatus has yet been found.

In the patients dying of *3-β-hydroxysteroid-dehydrogenase* deficiency the *adrenal* changes very much resemble those of the virilizing form

with severe salt loss. The hyperplasia is more marked in the fatal cases so far examined.

In the two studied cases, there was no detectable histochemical activity of 3-β-hydroxysteroid-dehydrogenase, whereas the other enzymes of the adrenal cortex were active (SIEBENMANN, 1973). The enzyme activity of Leydig cells could not been investigated, since they had already been involuted in the infants examined up to now.

ε) Laboratory Data

The analysis of three particular steroid groups is important for precise differentiation of the different types of the congenital adrenogenital syndrome. These three groups are: androgens, pregnane (and pregnene) derivatives, and cortisol and its metabolites. Group estimation of 17-ketosteroids (see p. 381) is often not sufficient for differentiation. The individual 17-ketosteroids can be assayed by gas chromatography. This is especially important in differentiation between a 21-hydroxylase and an 11β-hydroxylase defect. Estimation of testosterone in the plasma or urine is also of significance (see below). Among the *pregnane derivatives*, pregnanetriol and particularly pregnanetriolone have become important since only small amounts of pregnanetriol and no pregnanetriolone at all appear in the urine of normal subjects. Increased amounts excreted in the urine are therefore of pathognomonic significance. *Pregnene derivatives* such as Δ5-pregnenediol, Δ5-pregnenetriol and Δ5-16-α-hydroxypregnenolone (in infants) are also of significance, although they are more difficult to identify. Estimation of cortisol and corticosterone precursors tetrahydro-S and tetrahydro-DOC is also important in differentiation between different forms of congenital hyperplasia of the adrenal cortex (see below). The reader is referred to p. 379 for further details about the methods of estimating steroid hormones. Apart from the methods described in that section, *gas-chromatographic* methods have proved particularly suitable for the measurement of testosterone, pregnane derivatives and individual 17-ketosteroids.

ζ) Clinical Features

This section presents the clinical pictures and steroid findings in the three main forms of congenital hyperplasia. Since the clinical picture can vary considerably depending on the type of enzyme defect, a generalized account of the clinical picture in the adrenogenital syndrome is not given.

η) 21-Hydroxylase Deficiency

This is the most common type of congenital hyperplasia of the adrenal cortex, accounting for about 90% of cases. Cases with *salt loss* and those where there is no salt loss differ pathogenically only in the degree of the enzyme defect. However, a few authors postulate that there are two different 21-hydroxylases (see

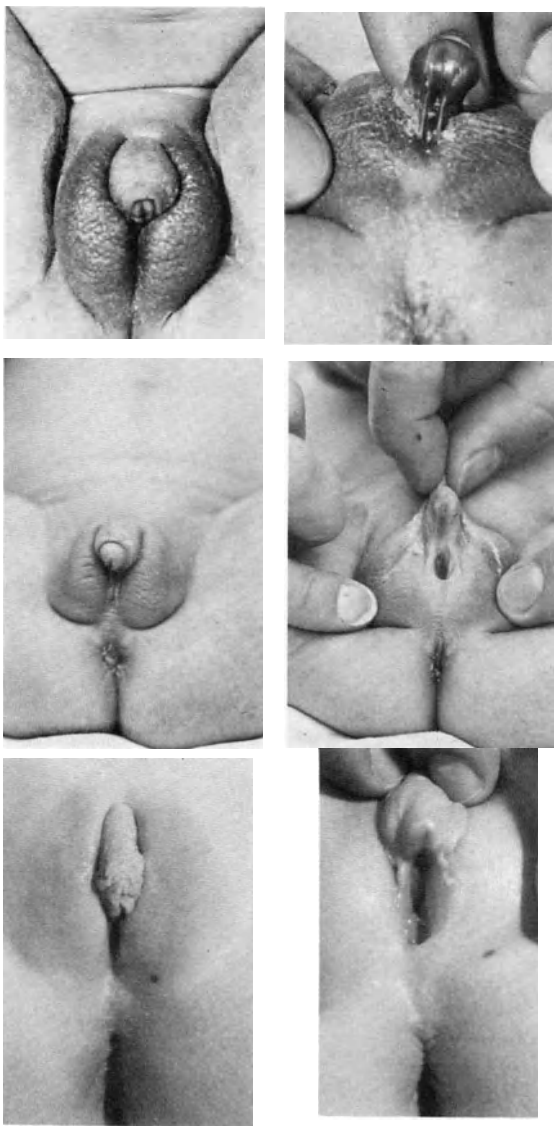


Fig. 29. External genitalia of 3 girls with congenital adrenogenital syndrome. Different degrees of virilization. Above: markedly enlarged clitoris with open urethral groove at the ventral side of the clitoris, small urogenital orifice (sinus urogenitalis) on the ventral basis of the clitoris and scrotum-like labia majora, corresponding to genital type IV in the scheme, p. 710. Middle: identical findings but larger urogenital orifice (sinus urogenitalis), corresponding to genital type III. Below: moderately enlarged clitoris with almost normal vulva with separate urethral and vaginal orifices, corresponding to genital type I

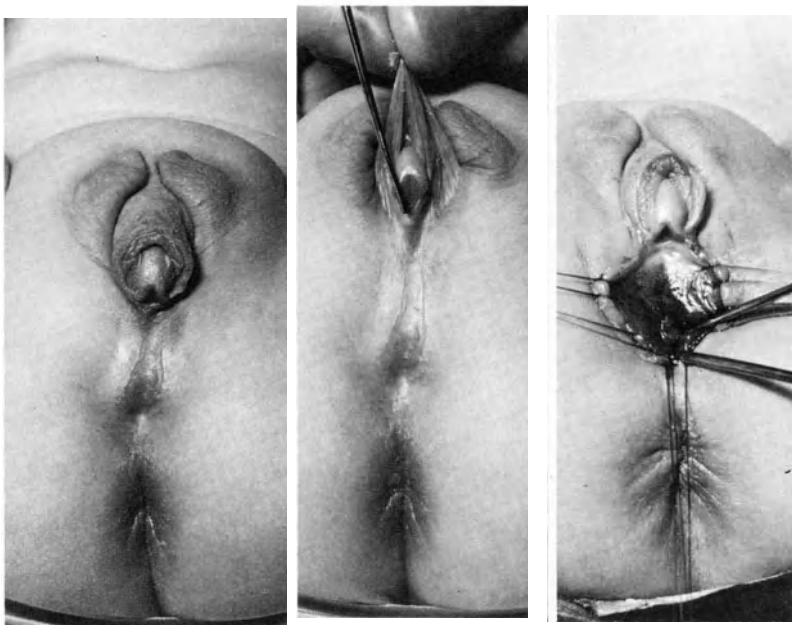


Fig. 30. Genitalia of a 3-year-old girl with congenital adrenogenital syndrome. Genital type IV according to scheme, p. 710. The superficial sinus urogenitalis can be recognized from an external flat groove. The internal urethral orifice (thin probe) and the vaginal orifice (thick probe) are localized where it ends in a pit (left side and middle)

below). In the *form without salt loss* (simple virilizing adrenal hyperplasia), *androgenic symptoms* are predominant. *Developmental anomalies of the external genitalia in girls* are the only effects caused by androgen overproduction in the fetus (PRADER, 1954). While the internal genitalia are unaffected, the external genitalia show masculine features under the pathologic action of androgens between the 12th and 16th weeks of pregnancy. The genitalia therefore have a more or less ambiguous appearance even in the newborn, and the term female *pseudohermaphroditism* is then applied. The clitoris is enlarged like a penis, is erectile, and continues to grow in later years if left untreated. An urogenital groove can often be seen on the ventral side (Fig. 29). The labia majora develop to be scrotum-like and fusion may even occur. The labia minora are usually completely absent. Instead of separate orifices for the urethra and vagina, usually only a single funnel-shaped or urethra-like urogenital opening is found on the ventral basis of the clitoris (Figs. 29 and 30). In addition to these classic findings, which are the most frequent, there are also all intermediary forms, ranging from slight enlargement of the clitoris with a normal vulva and distinct labia minora, to complete masculinization of the external genitalia (STOLECKE, 1970). A prostate anlage is frequently found, but otherwise the *internal genitalia* are always feminine. Urethroscopy and retrograde urethrography with contrast medium show how the urethra-like urogenital sinus divides into vagina and urethra (Fig. 30). The urogenital sinus is situated quite superficially, and can be recognized on external examination by a flat groove. This groove runs towards the anus and terminates in a small depression behind which the internal urethral and vaginal openings lie (Fig. 30). The genital changes can no longer be influenced even if treatment is instituted early. In contrast to the female genitalia, the *male genitalia*

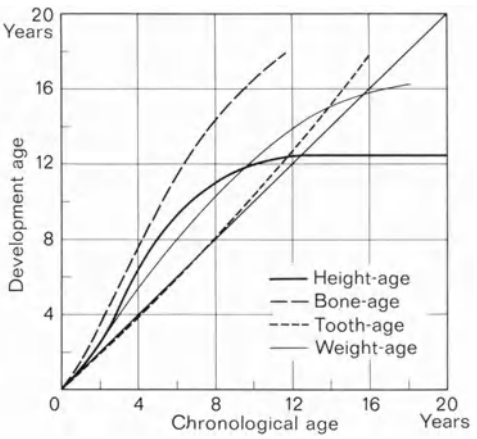


Fig. 31. Mean course of length, weight, bone and tooth development in congenital adrenogenital syndrome. Note the growth acceleration, the precocious arrest of growth and the discrepancies between the accelerated bone development and the normal tooth development

develop in an absolutely normal manner. The penis alone may be strikingly enlarged even at birth, but it is more common for enlargement to occur during the first few years of life. The enlargement increases rapidly in untreated patients in subsequent years, whereas the testes usually remain small. The genital region is often strikingly pigmented.

Accelerated increase in height and bone maturation are the next androgenic symptoms to occur in untreated patients. Weight, height and bone development are normal in these patients at birth. Towards the end of the first year or later, acceleration in bone maturation can be recognized radiologically. This is rapidly followed by a striking acceleration in height. Within the next few years, height and bone age are considerably ahead of chronological age (Fig. 31), bone age in turn being strikingly in advance of height. This results in premature fusion of the epiphyses at the age of about 10. Growth thus comes to a stop before normal adult height is reached. The patients are therefore of normal height in the first year, unusually tall between the 3rd and 10th years, and unusually short after the 13th year. The height of adult untreated patients is usually between 135 and 155 cm, and they are usually disproportioned (Fig. 34). Correctly treated patients are usually of normal height and their bone maturation (Fig. 35) is also normal.

Shortly after acceleration of growth and bone maturation become recognizable, the *secondary sexual characteristics* develop in untreated patients. Pubic hair appears between the 2nd and 5th year and axillary hair and gradually increasing body hair between the 4th and 7th years. The voice breaks at about the age of 10, when pubic and body hair become more masculine in appearance and facial hair increases (Figs. 32 and 33). Baldness may occur in the adult woman (Fig. 33).

Standstill of gonadal development contrasts with the premature appearance of the secondary sex characteristics. This developmental failure can be interpreted as hypogonadotropic hypogonadism since secretion of the pituitary gonadotropins is inhibited by the androgens (p. 452). In the girl, this causes failure of breast development and primary amenorrhea, and in the boy the testes remain strikingly small, contrasting with the marked development of the penis. Occasionally, however, normal testicular growth is observed, or the testis shows tumor-like enlargement. Biopsy may reveal hyperplastic adrenocortical tissue situated intratesticularly in these cases (HEDINGER, 1955; PIYARATN, 1957; EARLL, 1969). Boys with normal spermato-

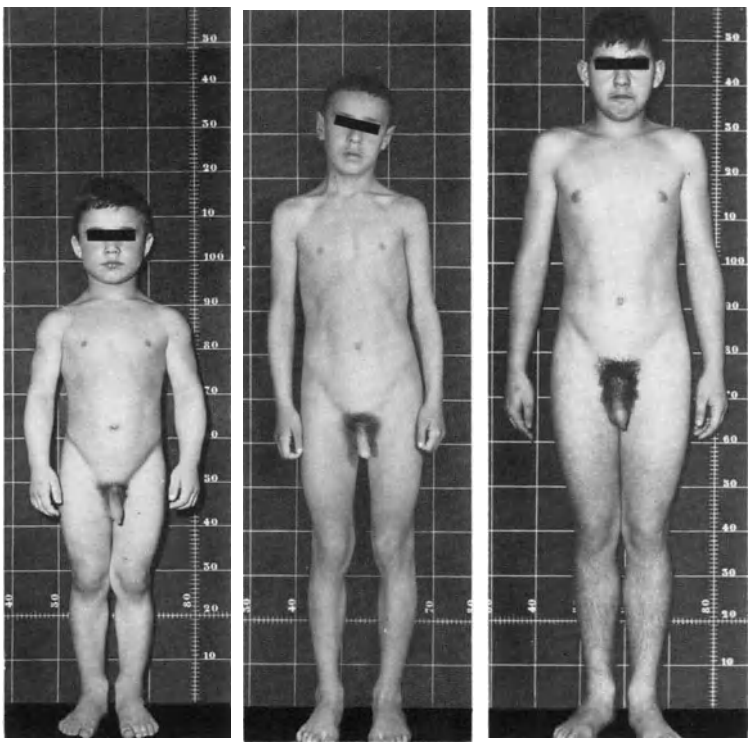


Fig. 32. Three boys with adrenogenital syndrome. The patient on the left has an adrenocortical tumor, the other two boys have adrenocortical hyperplasia

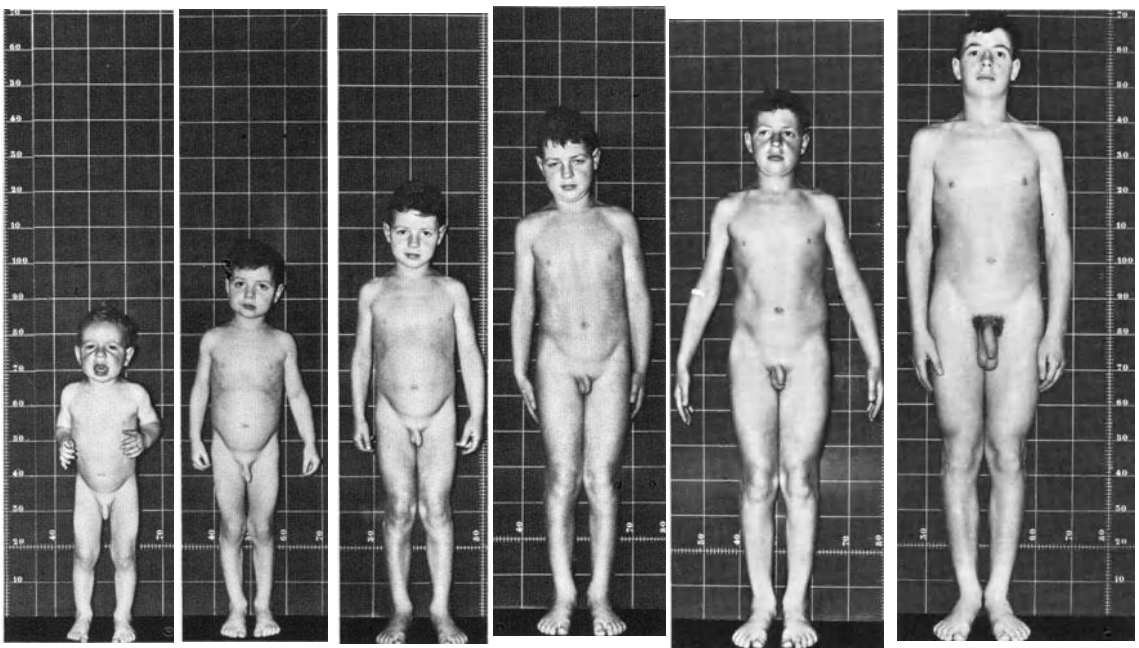


Fig. 33. Normal development and spontaneous puberty in a boy with congenital adrenogenital syndrome due to 21-hydroxylase deficiency, treated since infancy

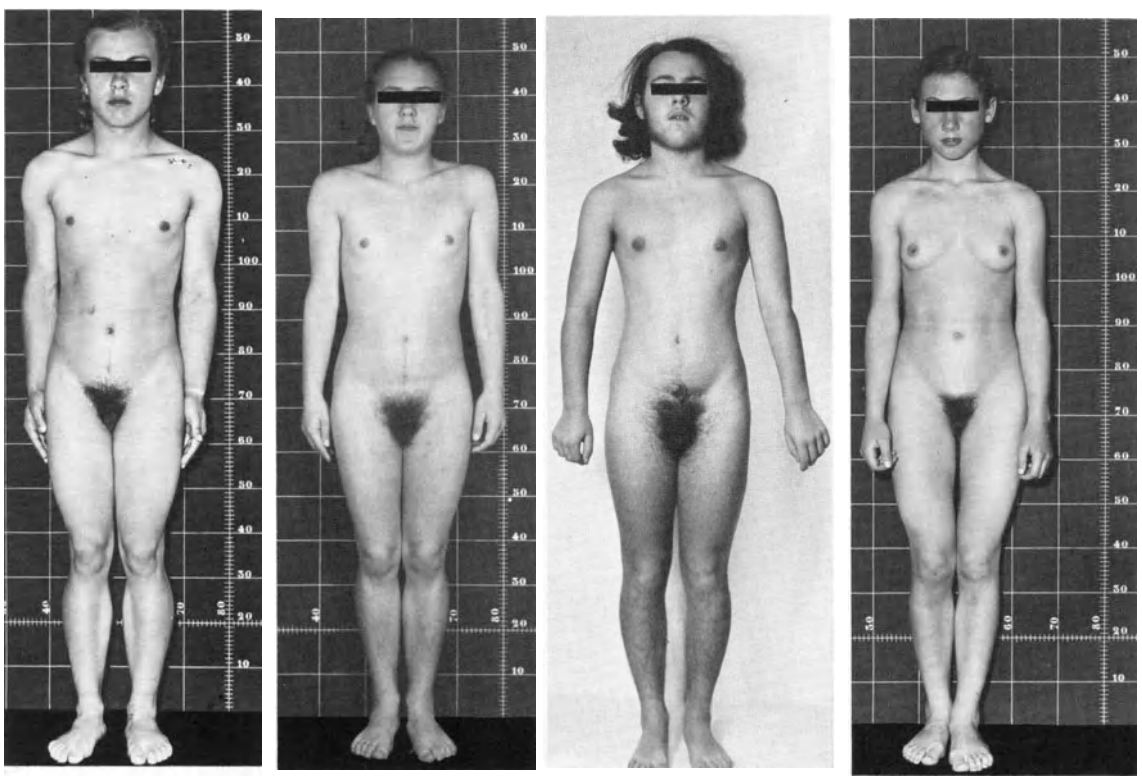


Fig. 34. 4 girls with congenital adrenogenital syndrome at the ages of 11 to 12 years. The three girls on the left are untreated. The girl on the right has been treated with cortisone for 3 years. Note the difference between the virilized untreated patients and the completely female development in body build and breast formation of the treated girl. (PRADER, 1956)

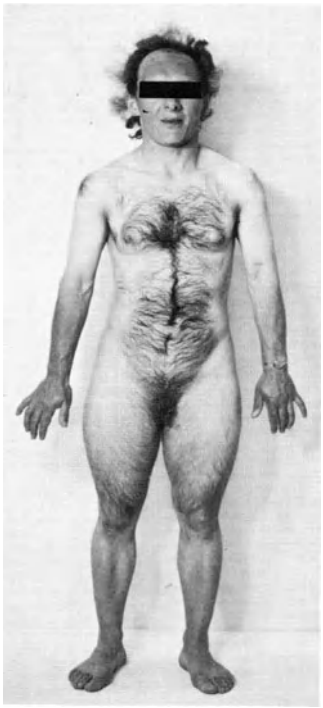


Fig. 35. Untreated 27-year-old woman, 145 cm tall, with congenital adrenogenital syndrome due to a 21-hydroxylase deficiency

genesis and girls with some slight development of the breasts and sporadic genital bleeding have also exceptionally been observed.

Thus, the premature physical development in untreated patients is not caused by true precocious puberty but rather by *precocious pseudopuberty* (see Chapter XIX). Since the precocity is of a masculine type in both sexes, it is described as isosexual in the boy and heterosexual in the girl. Normal gonadal development and normal spontaneous puberty are possible with treatment (Figs. 33 and 34).

Whereas the acceleration in growth and premature appearance of the male secondary sexual characteristics become obvious early in untreated cases, the *male stature* and *increased muscular development* usually appear later on and are more conspicuous in girls. The strong skeletal structure, wide shoulders, rather narrow hips and strong athletic musculature are particularly striking. The adult female patient looks like a small, thick-set but very strong man. This impression is further accentuated by the voice, facial features, growth of beard and baldness which occasionally arise (Fig. 35). The *skin* is not only more hairy but also thicker and coarser than that of a healthy woman. Acne occasionally occurs but is usually not as severe as in some healthy subjects during puberty. Brownish spots or a diffuse Addison-like pigmentation (see p. 316f.) are seen on the skin and less frequently on the mucosa in a few untreated cases.

In contrast to somatic development, intellectual, *psychic and psychosexual development* is not usually accelerated in untreated patients. The psychological picture is nonspecific and fits in with the general patterns of BLEULER's endocrine psychosyndrome.

The physical *efficiency* is usually not impaired. Most patients survive illnesses and surgery without complications. The *life expectancy* is hardly reduced. However, there are frequent reports of patients dying suddenly in a state of shock during mild infectious diseases such as measles or following an operation. Death in these cases is due to the inability to react to stress with increased glucocorticoid production.

The *steroid findings* can be deduced from Fig. 27. Increased amounts of steroids not hydroxylated in position 21 and their catabolites are found in the plasma and urine. Levels of 21-hydroxylated steroids are lowered. The total 17-ketosteroids increase steadily from an approximate mean value of 2–3 mg/24 h in the young infant up to a mean of about 40–50 mg/24 h or more at the age of about 15 in untreated patients. There are wide individual variations, and values can rise even higher in conditions of stress. Fractionation of the 17-ketosteroids shows that it is predominantly catabolites of androstenedione and dehydroepiandrosterone, namely androsterone and etiocholanolone which are excreted in the urine. Since 11β-hydroxylation is uninhibited in 21-hydroxylase deficiency, increased amounts of the corresponding 11-hydroxy-, and 11-keto-17 ketosteroids (11-hydroxyandrosterone, 11-hydroxy-etiocholanolone, 11-ketoandrosterone and 11-keto-etiocholanolone) are also excreted. 11-Hydroxylation of these 17-ketosteroids competes with 11-hydroxylation of the small amounts of substance S which are formed in spite of the deficiency (see Fig. 27). This leads to a further reduction in cortisol synthesis. Therefore, more tetrahydro-S (catabolite of S, see Fig. 27) is excreted in patients with 21-hydroxylase deficiency than in normal subjects, although significantly less than in patients with 11β-hydroxylase deficiency (see below). Excretion of dehydroepiandrosterone (principally as sulfate) is also elevated, but to a lesser degree. *Testosterone* arising in the adrenal cortex and periphery from androstendione and dehydroepiandrosterone, is also found to be raised in the plasma and urine (as glucuronide). This fact must be held primarily responsible for the clinical symptoms of the virilization (DEGENHART, 1965). Further,

estrogens (estrone and estradiol) arise from androstenedione or testosterone, and are also excreted in increased amounts (BONATI, 1968). Estriol has an exceptional position among the estrogens, since it is a 16-hydroxylated compound and develops mainly from 16α-hydroxy-pregnenolone.

Although the clinical symptoms are predominantly due to the androgens, the increase in progesterone and 17α-hydroxyprogesterone and their catabolites (steroids just "before" the block) is even more impressive. The plasma progesterone level is 6 to 10 times higher in children with 21-hydroxylase defect than in adult men, and plasma 17α-hydroxy-progesterone 50–200 times as high (STROTT, 1969). *Pregnanetriol* mainly arises directly from 17α-hydroxy-progesterone (HALPERIN, 1967). Its rate of secretion can be increased almost 100-fold (240–280 mg/24 h, normal: 3 mg/24 h) in cases of 21-hydroxylase deficiency. *Pregnanetriolone* differs from pregnanetriol in having an additional keto-group in position 11, and is not normally found in urine even after ACTH stimulation (FINKELSTEIN, 1962). Several mg are, however, excreted daily. In most cases, more pregnanetriol than pregnanetriolone is excreted, but there are cases where the situation is reversed, especially in young infants. This appears to be connected with a predisposition to 11-hydroxylation in early life. Other steroids found to be elevated are: Δ5-pregnenediol (from pregnenolone, see Fig. 27), Δ5-pregnenetriol (from 17α-hydroxy-pregnenolone) and 16α-hydroxy-pregnenolone (from pregnenolone) which can be further metabolized into estriol (REYNOLDS, 1965; JANOSKI, 1969). Reduction in the excretion of 17-hydroxycorticosteroids (Porter-Silber chromogens) can reflect the fall in the rate of cortisol production. However, cortisol production is frequently normal but does not rise or rises only slightly in response to ACTH. Individual cortisol metabolites and plasma 11-hydroxy-corticoids behave in a similar manner. However, it must be remembered at this point that increased amounts of 11-hydroxyandrostenedione and 21-desoxycortisol (which are measured at the same time by the nonspecific fluorometric method; see p. 380) can simulate a normal or even a raised cortisol concentration, while cortisol itself is in fact reduced. *Aldosterone* secretion is normal or even raised in patients with 21-hydroxylase deficiency without salt loss (PRADER, 1955; BLIZZARD, 1959; BONGIOVANNI 1968; GODARD, 1968). BARTTER (1968) has concluded from this that 21-hydroxylation is intact in compounds not hydroxylated in position 17 (see Fig. 27), thus permitting normal aldosterone production. If this is so it must be assumed that two different 21-hydroxylases exist in the human adrenal cortex (DEGENHART, 1965). The increased aldosterone secretion would then have to be interpreted as compensation for the increased production of natriuretic steroids in patients without the salt-losing syndrome (see below).

The typical steroid changes are sometimes only found after stimulation with ACTH in newborns and young infants with 21-hydroxylase deficiency (p. 372).

The most important steroid findings in 21-hydroxylase deficiency are summarized in Table 17.

Azoospermia is found in the untreated male, and testicular biopsy reveals immature tubules and only immature Leydig cells or none at all. These findings may be due to hypogonadotropic hypogonadism, but they are not constant and cases with normal spermiogenesis have been reported. Surprisingly, the vaginal smear of an untreated female shows no signs of estrogen activity, although chemical methods of measuring urinary estrogens show that elevated amounts are excreted in both sexes.

9) 21-Hydroxylase Deficiency with Salt Loss

This may be due to a deficiency of the same enzyme as simple 21-hydroxylase deficiency in a more pronounced form. It is, however, possible that 21-hydroxylase, which is responsible for the conversion of progesterone into DOC (see Fig. 27), is intact in the simple form and absent in the form with the salt-losing syndrome (see above).

A clinically manifest *electrolyte disturbance* similar to that in Addison's disease is found in some patients with 21-hydroxylase deficiency. This is characterized particularly by renal loss of sodium. It is common during infancy, less frequent during late childhood, and rare during adulthood. This age distribution may very well be due to the fact that patients instinctively learn to eat more salt. In addition, however, there is probably a tendency for the salt-losing syndrome to improve with increasing age. It is doubtful whether there are any geographic differences in the incidence of the salt-losing syndrome. HIRSCHFELD (1969) found an accumulation in Alaskan Eskimoes, MARKS (1969) found more cases than CHILDS (1956) in Maryland. It must be remembered, however, that more recent work cannot be compared to earlier statistics without certain reservations. More sophisticated methods of diagnosis also detect more cases of the salt-losing syndrome.

The symptoms of salt loss usually arise during the first few days or weeks of life. They usually

Table 17. Results of steroid determinations in congenital adrenogenital syndrome with 21-hydroxylase deficiency (enzyme deficiency No. 1 in Fig. 27), altered steroids and their plasma and urinary metabolites

	Adrenocortical secretion	Plasma concentration		Urinary excretion	
Increased					
Androgens	Dehydroepi-androsterone Androstenedione Testosterone	Androsterone Dehydroepi-androsterone Androstenedione Testosterone	Sulfate	Total 17-ketosteroids Dehydroepiandrosterone sulfate Androsterone 11-Hydroxyandrosterone 11-Ketoandrosterone Etiocholanolone 11-Hydroxyetiocholanolone 11-Ketoetiocholanolone Testosterone glucuronide	Glucuronides + Sulfates
Pregnane derivatives	17-hydroxy-progesterone	17-hydroxyprogesterone		Pregnanetriol Pregnanetriolone (= 11-ketopregnanetriol)	Glucuronides
Estrogens	Estrone Estradiol Estriol			Estrone Estradiol Estriol	
Decreased					
Cortisol and metabolites	Cortisol Corticosterone Cortexolone	11-Hydroxycorticoids[a] Cortisol Corticosterone Cortexolone (S)		Total 17-Hydroxycorticoids Tetrahydrocortisol (THF) Tetrahydrocorticosterone (THB) Tetrahydrocortisone (THE) Tetrahydrocortexolone (THS)[b]	

[a] Often increased due to non-specific cross-reaction with 21-deoxycortisol and 11-hydroxyandrostenedione.
[b] Often slightly increased (p. 369).

arise gradually but occasionally develop suddenly. The most common symptoms are anorexia, lethargy, inadequate weight gain, vomiting and frequent mild diarrhea. Vomiting is almost always present, usually occurring in a single stream, sometimes spastic in nature with visible gastric peristalsis. These symptoms of chronic salt loss are often followed by true crises, usually in association with an infection (stress). Without correct treatment, death may result. These crises are characterized by acute loss of weight, severe dehydration, and shock. If these patients survive, whether spontaneously or as a result of treatment, they often demonstrate a pronounced craving for salt towards the end of the first year or often even before. The symptoms disappear completely in some patients after 1–3 years, but the salt-losing syndrome can become manifest again at any time during intercurrent illnesses in early infancy.

Apart from symptoms due to salt loss, all other symptoms of 21-hydroxylase deficiency without salt loss can also occur in these patients. The only difference is that growth and bone development are retarded during the first year or two years due to the severely impaired electrolyte metabolism, and the characteristic acceleration does not occur until later.

Pathogenesis of the salt-losing syndrome can be explained in part by a relative aldosterone deficiency. In patients with severe salt loss, aldosterone and its metabolites are excreted in reduced amounts and the aldosterone production rate is decreased (BRYAN, 1965). Limitation of the sodium intake almost always induces a poor aldosterone increment in the salt-losing syndrome (BONGIOVANNI, 1967). Thus, apparently due to the enzyme deficiency, not enough aldosterone can be synthesized. This interpretation is also supported by the elevated plasma renin activity (IMAI, 1968) and the hypertrophic juxtaglomerular cells in patients with the salt-losing syndrome. The fact that infections and ACTH promote sodium loss and glucocorticoids inhibit it suggest that there are other pathogenic factors. In fact, there is evidence that progesterone and 17α-hydroxyprogesterone as well as other unknown steroids exert an antagonistic effect on the action of mineralocorticoids on the renal tubules; in other words, these steroids have a natriuretic action. This would explain why a compensatory increase in aldosterone excretion is sometimes found in patients with simple 21-hydroxylase deficiency without the salt-losing syndrome (see p. 369). It has recently been shown that 16α-hydroxy-progesterone has a natriuretic action. Since 16-hydroxylated compounds are found mainly in infants, this may explain the tendency towards spontaneous regression of the salt-losing syndrome with increasing age (JACOBS, 1969).

ι) 11-Hydroxylase Deficiency

There are few cases of congenital hyperplasia of the adrenal cortex in which an increase in blood pressure is the dominant clinical feature arising in association with other symptoms due to androgens. These symptoms may be as severe as or less pronounced than in cases with 21-hydroxylase deficiency. A salt-losing syndrome never arises. As in the other forms, the disorder is uniformly present or uniformly absent in siblings of the patient affected. The hypertension, like the salt-losing syndrome, is worsened by ACTH and improved by glucocorticoids. These cases are due to deficiency of 11β-hydroxylase, which converts substance S into cortisol and DOC into substance B (corticosterone) (Fig. 27). The steroid findings are therefore different from those found with 21-hydroxylase deficiency: androgens are similarly raised but to a lesser extent. On the other hand, 11-hydroxy-17 ketosteroids are absent or their excretion is greatly reduced. The excretion of *pregnanetriol* is also raised in this condition, but not as strikingly as in 21-hydroxylase deficiency. *Pregnanetriolone* is absent or present only in small amounts. In contrast, more substance S and DOC are produced and this is reflected in a greatly increased excretion of their metabolites tetrahydro-S and tetrahydro-DOC. Accumulation of the mineralocorticoid DOC (which is, however, less active than aldosterone) explains the hypertension and absence of a salt-wasting syndrome. Cases without hypertension have, however, been reported, as have cases with hypertension but no increase in tetrahydro-DOC excretion. Nevertheless, it must be remembered that in young infants, S and DOC are not only metabolized into the corresponding tetrahydro compounds but can also be excreted directly. Thus, low levels of tetrahydro-S and DOC in the urine of a baby in the first month of life does not exclude the diagnosis of an 11β-hydroxylase defect. The concentrations of plasma 11-hydroxycorticoids and urinary 17-hydroxycorticoids are greatly increased provided they are measured by nonspecific methods (p. 380). This increase is, however, not due to a rise in cortisol but to substance S. The rate of secretion of aldosterone is reduced (KOWARSKI, 1968). Plasma renin is also lowered (probably suppressed by DOC; IMAI, 1968).

Recently, a case of this deficiency has been described where only the 11β-hydroxylation of S to cortisol, but not that of DOC to corticosterone was impaired (ZACHMANN, 1971). This suggests that two 11β-hydroxylating systems exist in the human adrenal.

Metyrapone represents a model of 11β-hydroxylase deficiency. This substance, which is used in examination of the ACTH reserves of the anterior pituitary lobe (see p. 387), temporarily blocks 11β-hydroxylase.

κ) 3β-Hydroxysteroid-Dehydrogenase Deficiency

This form of congenital hyperplasia of the adrenal cortex differs from the forms described above in that there is only a slight production of biologically active androgens, so that virilization may be absent or only mild. In the rare cases of deficiency of 3β-hydroxysteroid-dehydrogenase, steroid synthesis is blocked at the stage of the Δ5 compounds (pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone; see Fig. 27) (BONGIOVANNI, 1961, 1962). Thus dehydroepiandrosterone is the only androgen produced in excess. All the other steroids excreted in increased amounts are also Δ5 compounds, namely Δ5-pregnenediol (from pregnenolone), Δ5-pregnenetriol (from 17α-hydroxypregnenolone) and, depending on age, also 16α-hydroxy-pregnenolone. Since 21-hydroxylase is intact in these cases, Δ5-pregnenetriol, for example, can still be hydroxylated in position 21, resulting in Δ5-pregnenetetrol, a substance which BONGIOVANNI (1962) has demonstrated in a few cases. Pregnanetriol is absent or, with partial enzyme deficiencies, reduced or normal, but is not increased. Pregnanetriolone, testosterone and tetrahydro-S are absent. Since dehydroepiandrosterone is not very active biologically and cannot be converted into androstendione or testosterone (Fig. 27), only mild virilization arises in this syndrome if any at all. Male patients have incompletely masculinized genitalia (various degrees of hypospadia or phenotypic female genitalia). This can be explained by the absence of 3β-dehydrogenase in the testes as well and by their failure to form the testosterone necessary for normal development of the male genitalia during organogenesis. In contrast to the situation in 21- and 11β-hydroxylase deficiencies, the genitalia in female patients are normal or virilization is only slight, much less pronounced than in the other types. In most of the known cases there is also a severe salt-losing syndrome (p. 369f.). 3β-Dehydrogenase deficiency usually appears to be more complete than the other enzyme defects described. In the majority of cases, no cortisol metabolites at all are found in the urine. This would explain why patients with this syndrome almost always suffer from Addison-like crises and why most of them die in the first months of life despite adequate treatment. *Lipoid hyper-*

plasia of the adrenals (20, 22 desmolase defect, Fig. 27) is described in another section (p. 323).

17-Hydroxylase deficiency (Fig. 27) is connected with overproduction of corticosterone and desoxycorticosterone and is therefore also described in another section (p. 339).

λ) Diagnosis and Differential Diagnosis

Diagnosis of the different forms of the congenital adrenogenital syndrome is based on the family and personal histories, typical clinical features and characteristic steroid findings.

There is often a family history of sisters with intersexual genitalia or prematurely developed brothers. The differential diagnosis includes male pseudohermaphroditism in girls (p. 724) and true precocious puberty in boys (p. 1046).

In taking the *personal case history*, it is important to establish in girls whether the ambiguous genitalia were present at birth or whether they became obvious only later. In boys, the time at which penis enlargement was first noticed must be determined.

Clinical examination reveals the typical findings described above. Obesity and other symptoms of CUSHING'S disease do not belong to the classic picture but rather suggest a tumor of the adrenal cortex. In addition to the steroid findings, the genital findings and the degree of development of the secondary sexual characteristics are decisive for the diagnosis, although the possibilities in the differential diagnosis vary with the sex. A raised specific steroid excretion is characteristic of each type of enzyme defect. This steroid excretion can be normalized by glucocorticoids. This contrasts with the situation in the adrenogenital syndrome due to a tumor of the adrenal cortex, where the greatly increased excretion of 17-ketosteroids (dehydroepiandrosterone in particular) is unaffected by glucocorticoids (e.g. dexamethasone, see p. 389).

In *female patients* with a 21- or 11β-hydroxylase defect, the overproduction of androgen can be recognized immediately, suggesting a disorder in the adrenals or ovaries. The genital changes with the typical urogenital sinus permit the exclusion of "premature pubarche" (p. 1040) and prove the congenital nature of the disorder. The other types of intersexuality to be considered can be excluded during the first years of life by demonstration of chromosomal female sex and the presence of increased amounts of androgens. A case with hypertrophy of the clitoris without a urogenital sinus can be differentiated from androgenic tumors of the adrenal cortex and ovary by the steroid findings. A normal female fetus has been exposed to androgens. This occurs when the mother receives gestagens containing androgens or steroids which can be metabolized into androgens during pregnancy. In rare cases, virilization of the fetus (or even complete masculinization) has been observed due to an ovarian tumor (arrhenoblastoma) or a virilizing adrenocortical adenoma in the mother. Steroid findings, however, are normal in the children in all these cases and virilization does not progress after birth. Finally, forms of female pseudohermaphroditism associated with malformations (particularly of the kidneys) and probably not due to hormonal factors must also be considered in the differential diagnosis.

Isosexual development in the *male sex* (with the exception of 3β-hydroxysteroid-dehydrogenase deficiency) makes diagnosis very difficult. In the newborn, the penis is often of normal size or only slightly enlarged and the scrotum is sometimes only slightly pigmented. The testes are usually small in comparison to the general somatic development. They are seldom of normal size and are sometimes enlarged into tumor masses (p. 366). In true precocious puberty, the tests are usually of normal size. In contrast to 21- and 11β-hydroxylase deficiencies, the biopsy in a case of precocious puberty reveals Leydig cells and spermatogenesis, and the total 17-ketosteroids are not raised or only slightly. The same is true of "premature adrenarche" (p. 1040), where acceleration of somatic development is also absent. In cases with tumor-like enlargement of the testes, it is often difficult to differentiate congenital adrenocortical hyperplasia from a Leydig cell tumor, on the basis of a testicular biopsy. Differentiation is, however, possible on the basis of these findings: the tumor can only decrease in size and steroid excretion can only be normalized in a case of adrenocortical hyperplasia treated with glucocorticoids.

The symptoms are usually insignificant in the *adult man*. Mild forms of 21-hydroxylase deficiency can only be detected by taking an exact case history and performing routine steroid estimations in every case where the testes are unusually small, in cases with testicular tumors, sterility, and azoospermia, and in cases of small stature with full development of the secondary sexual characteristics.

An early diagnosis is of utmost prognostic importance, since treatment started early enough can bring about absolutely normal physical development and prevent salt-losing crises. Characteristic steroid findings may be absent in the young baby. If there is clinical suspicion of an adrenogenital syndrome or the total 17-ketosteroids are even only slightly elevated,

a provocation test with ACTH should be performed. As a rule, 1 mg/m^2 body surface of a synthetic depot ACTH is sufficient to produce the typical steroid findings. The 24-hour urine is collected the day before the test, the day the injection is given, and the following day. In the presence of 21-hydroxylase deficiency with the salt-losing syndrome or 3β-hydroxysteroid-dehydrogenase deficiency, this test is dangerous, since it may induce a potentially fatal salt-losing crisis. Glucocorticoids, mineralocorticoids and saline should be kept ready for intravenous administration.

When a congenital adrenogenital syndrome is suspected (affection of siblings) it may be possible to make the diagnosis *in utero* (CATHRO, 1969). The *estriol* excretion is raised in the urine of pregnant women from the 20th week of pregnancy when the fetus has 21-hydroxylase deficiency. This phenomenon is due to the fact that the excessive amount, of 16α-hydroxypregnenolone (p. 369) produced by the fetus are metabolized by the mother and the placenta into estriol. Theoretically, an even higher estriol excretion is to be expected in cases with a 3β-hydroxysteroid-dehydrogenase deficiency.

The differential diagnosis of *21-hydroxylase deficiency with the salt-losing syndrome* is particularly important in newborns. The free interval during the first few days or weeks, the weight loss, vomiting and the often visible gastric peristalsis usually suggest the much more common condition, pyloric stenosis. This diagnosis is contradicted by the rapid emptying of the stomach in the radiological investigations, frequent mild attacks of diarrhea, and the electrolyte findings. Occasionally the syndrome can also be mistaken for infectious gastroenteritis. A history stating that siblings were of indeterminate sex as babies, and that they suffered from pyloric stenosis or diarrhea before death occurred is very suggestive. The diagnosis is, however, confirmed by the steroid findings and electrolyte investigations: serum sodium and potassium are reduced in pyloric stenosis, whereas hyponatremia and hyperkalemia are found in 21-hydroxylase deficiency.

μ) Treatment

The only satisfactory basic treatment of all forms of congenital hyperplasia of the adrenal cortex is life-long *treatment with cortisol* or some other glucocorticoid. Cortisol acts in two ways: it inhibits the increased ACTH secretion, causing a reduction of the steroids accumulated "before" the enzyme defect, and it compensates for the inadequate endogenous production of cortisol. Treatment is thus both inhibitory and substitutive. It is also assumed that the treatment removes any possible inhibition of gonadotropin secretion. In any case, normal gonadal maturation is possible with adequate treatment.

In principle, the *lowest doses* resulting in the disappearance of symptoms should be chosen. This is important for the following reasons: Cushing's syndrome arises only after massive over-dosage, but a dose which is even very slightly too high and causes no other external clinical signs can lead to definite retardation of growth (BERGSTRAND, 1966; RAPPAPORT, 1968). This inhibitory action of cortisol on growth has also been observed in other conditions (e.g. asthmatics treated with steroids, or patients with rheumatoid arthritis or in pituitary dwarfism, see p. 98). This effect is due primarily on a peripheral antagonism between cortisol and growth hormone, but also to inhibition of the release of growth hormone from the anterior pituitary. On the other hand it has to be considered that patients with congenital hyperplasia of the adrenal cortex cannot react to stress with an increase in endogenous cortisol production, or only inadequately. For this reason the dose sometimes has to be massively increased during periods of stress. The treatment, although simple in principle, places considerable demands on the physician, who should keep the dose as low possible while recognizing the requirements for increased doses of cortisol during periods of stress.

Since the active physiological steroid absent is cortisol and treatment is partly substitutive, cortisol is generally chosen in preference to other glucocorticoids. Prednisone, prednisolone and the long-acting methyl prednisolone are sometimes used. Even in small doses, however, these compounds seem to exert an inhibitory effect on growth. It is best to refrain from using other synthetic glucocorticoids (especially the fluoridized), since they offer no advantages and sometimes produce unpleasant side effects.

The rate of production of cortisol in normal children is about 12 ± 3 mg/m^2/24 h in both sexes, and the basic dose for long-term treatment should correspond roughly to this figure. An intramuscular dose of about 12 mg/m^2/24 h is therefore sufficient. With oral treatment it must be remembered that not all the cortisol taken is completely absorbed. If the dose is regulated according to the normal rate of production, the oral dose of cortisol must be somewhat higher (approximately 15–25 mg/m^2/24 h) (MIGEON, 1968). This dose is lower than that recommended so far by most authors. They suggest a dose of about 35 mg/m^2/24 h. When prednisone is used, a quarter of this dose is

sufficient. Division of the daily dose over the day also seems to be of importance, but this point has not been thoroughly investigated. Theoretically, an effort should be made to imitate the physiological daily variations in the plasma level of cortisol (p. 300). It would therefore be better not to divide the recommended dose equally throughout the day, but to give three-quarters of the daily dose early in the morning and the remaining quarter in the afternoon.

The doses given are for long-term therapy. Initially, more (about twice as much) must be given for a few days or weeks.

It seems to be important to keep the dosage low during the period of growth, and particularly during the first two years of life. This is essential since only the optimal dose and early onset of treatment can ensure completely normal physical development. For this reason, *regular follow-up* examinations with monitoring of steroid excretion and any necessary correction of the dose of cortisol are important. Total 17-ketosteroids should be measured every few days in the first few weeks and later at intervals of a few weeks. The other steroids (particularly pregnanetriol and pregnanetriolone (in cases of 21-hydroxylase deficiency) should be checked every few months. It must be kept in mind during this procedure that not all steroid findings become normalized even with adequate treatment: in 21-hydroxylase deficiency, the total 17-ketosteroids and their fractions return completely to within normal ranges, whereas urinary pregnanetriol usually remains slightly elevated and pregnanetriolone continues to be demonstrable in the urine. A minimal excretion of pregnanetriolone is if anything desirable, as an indicator that the cortisol dose is not too high.

In addition to steroid excretion, body weight, blood pressure, growth, bone age and the secondary sex characteristics must be checked regularly, and menstruation should be exactly charted.

Once treatment has been stabilized, the dose can usually remain the same for years in adult patients. During the phase of growth, however, the dose must be adjusted to the increasing body surface.

Therapeutic success varies with the age and severity of symptoms at the onset of treatment and with the type of enzyme deficiency. Completely normal development can be attained in the *baby* with 21-hydroxylase deficiency (Fig. 33). In the *infant* and *school-age child*, acceleration of growth and bone development already in progress can be brought to a stop and physical development can be at least partly normalized. Masculine secondary sexual characteristics remain stationary until bone age reaches normal pubertal age (in girls at about 11, in boys at 13 years, p. 1024). At this point, cortisol treatment results in gonadal maturation and normal development of the isosexual secondary sexual characteristics before normal pubertal age is reached. In this way, breast development and menarche can take place in girls even before the 7th year, and testicular development and spermatogenesis in boys. Tumor-like enlargements of the testes can be reduced in size soon after the onset of treatment regardless of the age of the patient. This is due to the action of cortisol, which causes atrophy of the intratesticular adrenocortical tissue.

Without treatment the epiphyses fuse after the *10th year* of life, so that it is then impossible to influence growth, and the masculine stature, enlargement of the clitoris and the masculine voice in female patients can be affected only to a slight degree. Gonadal maturation and regression of body hair can still be expected in female patients, however. The breasts develop within a few months and menarche occurs. Facial and body hair usually regress only several months later. In male patients, the external characteristics remain unchanged apart from maturation of the testes.

Menstruation induced by treatment is more or less regular and usually due to a true ovulatory cycle. Frequency and duration of the bleeding are often the best indications of correct cortisol dosage. Spermatogenesis and pregnancy confirm the *fertility* arising in response to treatment.

During long-term cortisol treatment, *ACTH* stimulation and *intercurrent infections* induce a rise in steroid excretion in the urine. Thus, the adrenals remain capable of reacting in spite of partial atrophy, even if not to the same extent as before treatment. Since regular cortisol intake cannot cover a sudden increase in glucocorticoid requirements, it is advisable to increase the cortisol dose temporarily during any serious intercurrent illness, and particularly for any surgical intervention. There is now more reluctance to increase the dose, since mild infections with slightly raised temperature do not necessarily require an increase in dosage. The dose must be temporarily doubled in severe stress. The dose given should only be tripled in cases of severe infection and for operations.

Discontinuation of the long-term treatment is hardly ever indicated. If it were discontinued, temporary adrenocortical insufficiency and a gradual return of symptoms would be expected.

Malformation of the external genitalia is the only symptom which can no longer be

influenced by cortisol at any age. At the most, cortisol can inhibit further growth of the clitoris in the small girl. *Surgical correction of the external female genitalia* is therefore always indicated except in cases where the changes are minimal. Whenever possible, the procedure should be undertaken during babyhood or early infancy, before the child becomes aware of her genital anomalies and this has an untoward psychological effect. The operation should only be carried out under increased doses of cortisol and, in patients with the salt-losing syndrome, only when the serum electrolytes are normal. The clitoris can be excised or merely amputated. The latter procedure is adequate in most cases. An incision can be made into the superficial urogenital sinus, and the sinus can be exposed as far as the junction of urethra and vagina. A thorough genital check-up is necessary shortly before puberty so that a too-narrow vagina can be surgically corrected.

Success with the treatment mentioned is especially satisfactory in 21- and 11β-hydroxylase deficiency. Stabilization is more difficult in 3β-hydroxysteroid-dehydrogenase deficiency and crises due to salt loss can also arise frequently even during treatment.

There are basically two possible *treatments for the salt-losing syndrome*. 1. Covering sodium loss by increased sodium intake. 2. Administration of sodium-retaining steroids. In addition, cortisol may also inhibit the production of natriuretic steroids. As soon as the diagnosis has been made, the first and most important measure is the immediate administration of *salt and fluid* in adequate amounts. If the condition of the baby is alarming, 500–800 ml physiological saline and 250–400 ml glucose solution are given intravenously in the first few days. As soon as the baby can drink, this form of treatment is replaced by adding about 3–5 g of salt to the usual daily food. During the subsequent days and weeks, the maintenance dose, which is lower than the initial dose, must be ascertained individually in each case on the basis of serum electrolytes and weight gain. The maintenance dose depends on the patient and lies between 1 and 6 g of salt daily. Children usually respond excellently to this therapy, even though steroid excretion remains elevated and serum potassium is often not normalized.

Administration of desoxycorticosterone acetate (DOCA, in a daily dose of 2–6 mg in oily solution i.m.) or aldosterone (20–300 γ daily) is also indicated during salt-losing crises and in severe cases. Instead of these two compounds, fluorohydrocortisone can be given (dose adjusted to individual, approximately 50–200 γ/m^2 daily). The salt augmentation decreases with this procedure. If salt administration is adequate and cortisol treatment is correct, long-term treatment with mineralocorticoids is hardly ever necessary before the 2nd or 3rd year of life. Long-term treatment with DOC may be necessary in the first years of life. Microcrystalline injections are then given every 3–4 weeks.

In addition to the treatment described above, cortisol treatment based on the principles given is also instituted in patients with the salt-losing syndrome. Salt requirements decline as a result of treatment with cortisol, due either to the reduction of natriuretic steroids or to the sodium-retaining effect of cortisol itself (though this is slight).

As has already been mentioned, salt and fluid requirements increase during *intercurrent infections*, even during common colds. Experienced mothers give their children salted tea during these periods, and the children usually accept it readily.

After 1 to 3 years, a cautious attempt can be made to reduce the dose of mineralocorticoids and the daily salt supplement. Ultimately, treatment can be discontinued. More intensive treatment of longer duration is usually required in cases of salt-losing syndrome in 3β-hydroxysteroid-dehydrogenase deficiency than in cases with 21-hydroxylase deficiency.

Hypertension in patients with 11β-hydroxylase deficiency requires no special treatment as a rule since it disappears with adequate cortisol treatment. It may be necessary to give an antihypertensive drug as well at the onset of treatment in severe cases.

c) Acquired Adrenogenital Syndrome during Infancy

The majority of cases of the adrenogenital syndrome during infancy are due to congenital hyperplasia of the adrenal cortex (p. 360) with a deficiency of 21- or 11β-hydroxylase. It is debatable whether congenital hyperplasia of the adrenal cortex can be latent, causing no symptoms at first and become apparent later in infancy as an acquired adrenogenital syndrome. Some authors support this view (Brooks, 1960; Mahesh, 1968). In general, however, the acquired adrenogenital syndrome is due to an *androgen-producing adrenocortical tumor*. This form of the adrenogenital syndrome is uncommon, but nevertheless more common in children than in adults.

The macroscopic and microscopic appearance of androgen-producing adrenocortical tumors is indistinguishable from that of tumors causing Cushing's syndrome, feminization or

no endocrine effects. Conclusions about the endocrine activity can, however, be drawn from the secondary symptoms, from the behavior of the cortex of the other adrenal gland and from the steroid findings. Thus, cortical atrophy of the other adrenal occurs considerably less frequently in cases of the adrenogenital syndrome due to an adrenocortical tumor than in Cushing's syndrome. The tumors appear to be morphologically identical. The data on cortical tumors in the section on Cushing's syndrome (p. 330 and 357) is thus also applicable to androgen-active tumors. Carcinomas are more common than adenomas in this group, but they too are difficult to differentiate and occasionally they can only be definitely confirmed by the formation of metastases.

Broadly speaking, the androgenic symptoms are similar to those found in congenital hyperplasia of the adrenal cortex with 21- or 11β-hydroxylase deficiency (p. 371). It is of therapeutic importance to differentiate between these forms and an adrenogenital syndrome due to adrenocortical tumors. The former group is treated with glucocorticoids, whereas surgery is necessary when tumors are present. Differentiation on purely clinical grounds is often difficult. The same disorder in siblings and enlargement of the clitoris or penis at birth are only possible in congenital hyperplasia. In tumor cases the female genital findings are normal except for enlargement of the clitoris. In congenital hyperplasia a urogenital sinus is formed except in cases of 3β-hydroxysteroid-dehydrogenase deficiency and mild 21-hydroxylase deficiency. The salt-losing syndrome only occurs in certain forms of congenital hyperplasia (p. 369). In contrast to congenital hyperplasia, where glucocorticoid synthesis is reduced, *the adrenogenital syndrome is often associated with Cushing's syndrome* in cases with tumors.

Detailed steroid analysis is important for the differentiation. Estimation of the total 17-ketosteroids does not allow any distinction. Measurement of the pregnane derivatives and fractionation of the 17-ketosteroids (p. 370) are useful in the differentiation. Characteristic steroid findings (Table 17, pp. 368 and 370) are detected in the different forms of congenital hyperplasia. In cases of adrenocortical tumors resulting in acquired adrenogenital syndrome, pregnanetriol is almost never excreted in increased amounts and pregnanetriolone (p. 369) does not appear in the urine. On the other hand, the major part (about 90%) of the 17-ketosteroids excreted consists of dehydroepiandrosterone, which normally accounts for only a small part of the total 17-ketosteroids. Thus, the steroid findings in a tumor case are similar to those in congenital hyperplasia with 3β-hydroxysteroid dehydrogenase deficiency (p. 371). Cortisol or another glucocorticoid (dexamethasone, p. 389) promptly causes a fall in the elevated amounts of steroids excreted in congenital hyperplasia, whereas no effect or merely a slight one is produced in tumor cases, since hormone production is autonomous and largely independent of ACTH. As in Cushing's syndrome, pyelography, retropneumoperitoneum with tomography and oblique pictures, and possibly the aortography and renography can be employed for direct demonstration and localization of the tumor.

Surgical removal of the adrenocortical tumor is the only possible therapeutic measure. Since androgens do not inhibit ACTH, the adrenal cortex theoretically does not undergo atrophy in a case with an androgen-producing tumor. This means there should be no fear of a postoperative adrenocortical insufficiency such as is encountered in cases of adrenocortical tumors with Cushing's syndrome. Unfortunately, in practice this is not always the case since tumors often produce not only androgens but also glucocorticoids. Thus, the same measures as in Cushing's syndrome are instituted for the removal of any hormone-active adrenocortical tumor as a precaution (p. 330 and 357).

Results are excellent if no metastases are present and the tumor can be removed easily. However, metastases occasionally arise 2–3 years later. The postoperative course of somatic development is normal in these children, but prematurely developed secondary sexual characteristics usually only partly regress. If bone development is greatly advanced, small stature in adulthood is inevitable. If bone development before the operation corresponds to normal pubertal age, sudden gonadal maturation can be expected after the operation, even if the chronological age does not yet correspond to normal pubertal age. Cortisol treatment has the same effect in cases of congenital hyperplasia.

d) Acquired Adrenogenital Syndrome in the Adult

This syndrome is due to acquired hyperplasia of the adrenal cortex or to an adrenocortical tumor. It occurs predominantly in women. Virilization is as the dominant clinical feature. In contrast to congenital hyperplasia of the adrenal cortex, the genitalia, with the exception of the hypertrophic clitoris, are normally developed. Body configuration is feminine and breast development is normal. Pubic, body and facial hair are masculine in type. Secondary amenorrhea arises only if virilization advances. Ovulatory cycles and fertility can, however, also remain

intact. The total 17-ketosteroids in the urine are increased in cases of acquired adrenocortical hyperplasia and tumors. As in the acquired adrenogenital syndrome in infancy, dehydroepiandrosterone is the predominant steroid in the urine. Testosterone concentrations in the plasma and urine are also elevated. Pregnanetriol excretion is normal and pregnanetriolone cannot be detected. This excludes congenital hyperplasia of the adrenal cortex with 21-hydroxylase deficiency (p. 369). The differential diagnosis includes the different forms of hirsutism (p. 377) (although these are not usually accompanied by hypertrophy of the clitoris), ovarian tumors (arrhenoblastomas, p. 638) and the Stein-Leventhal syndrome (p. 609). In virilizing ovarian tumors, testosterone production is also increased, but excretion of 17-ketosteroids is normal or only slightly raised. If testosterone and dehydroepiandrosterone are elevated in the plasma or urine an adrenal tumor must be sought first by the methods described (p. 376). If testosterone alone is raised an ovarian tumor is more likely. In some circumstances, a laparoscopy or laparotomy to inspect the ovaries or surgical exploration of the adrenals may be necessary before diagnosis is possible. Unnecessary laparotomies in cases of idiopathic hirsutism must, however, be avoided.

If an adrenal tumor is found it must be removed surgically. The same precautions must be taken as for the removal of an adrenal tumor in Cushing's syndrome (p. 330 and 357).

Pathologic anatomy: these tumors are more commonly carcinomas than adenomas. Morphological details and difficulties in histological differentiation between adenoma and carcinoma are described on p. 342.

Some cases are not true acquired adrenogenital syndromes but a mild form of congenital 21-hydroxylase deficiency. These cases are easily recognized from the steroid findings (pregnanetriol raised, pregnanetriolone present). Congenital 11β-hydroxylase deficiency can become manifest for the first time during adulthood (GABRILOVE, 1965). It becomes apparent in a much increased excretion of tetrahydro-S and tetrahydro-DOC. In addition, 17-hydroxycorticoids rise in the urine in response to ACTH in acquired hyperplasia or in tumor cases, whereas there is only a slight rise or none at all in congenital cases.

Substitution with glucocorticoids may be indicated after surgical removal.

e) Idiopathic Hirsutism

Hirsutism (p. 359) signifies an increase in sexual, body, and facial hair in women with no concomitant signs of virilization. There is no hypertrophy of the clitoris and the voice does not become deeper, or only to a slight extent. Some patients have menstrual disorders (oligomenorrhea, anovulatory cycles, secondary amenorrhea). Others menstruate normally and remain fertile.

Hirsutism in women is extremely common. Conditions to be excluded are: congenital hyperplasia of the adrenal cortex (p. 359), acquired adrenocortical hyperplasia or an adrenocortical tumor (p. 376), Stein-Leventhal syndrome and a virilizing ovarian tumor (p. 638). The case history, thorough gynecological examination, and steroid tests are useful in the investigation. If obesity is present as well, Cushing's syndrome must also be considered. Only when all these have been excluded can idiopathic hirsutism (i.e. caused by adrenal hyperactivity) be diagnosed.

Excretion of the 17-ketosteroids in the urine and the concentration of testosterone in the plasma are slightly raised in idiopathic hirsutism and in hirsutism due to ovarian production of androgens. Differentiation is often possible if urinary 17-ketosteroids and the plasma concentration of testosterone are examined after administration of ACTH (p. 385) and chorionic gonadotropin (during simultaneous treatment with dexamethasone (p. 389)). In idiopathic hirsutism, excretion of the 17-ketosteroids and the concentration of plasma testosterone increase more than is normal after ACTH. Dexamethasone has an inhibitory effect. Chorionic gonadotropin has only a slight effect or none at all. In contrast, in cases of hirsutism due to ovarian factors, urinary 17-ketosteroids and plasma testosterone rise most after chorionic gonadotropin, whereas ACTH and dexamethasone only have a weak effect.

Glucocorticoids often produce therapeutic success in idiopathic (adrenal) hirsutism. If, for example, cortisol is given in a dose which reduces urinary 17-ketosteroids to normal values (5–10 mg/24 h), menstruation is normalized and the hair gradually becomes less. Hirsutism due to androgens produced by the ovaries is not influenced by glucocorticoids but may decrease under estrogens and gestagens.

5. Adrenal Feminization

The term adrenogenital syndrome really includes all adrenal disorders causing overproduction of sexual hormones. It is customary, however, to use this term only in connection with the syndrome with overproduction of androgen, i.e. with virilization. Disorders with overproduction of estrogens are much more

uncommon and are described as adrenal feminization. The most common cause of this syndrome is a *feminizing adrenocortical tumor in the male.* About 70 cases have been reported up to now (GABRILOVE, 1965). Clinical and hormonal findings vary considerably. Often, carcinoma of the adrenal cortex is the cause. In Gabrilove's series, 41 of 52 patients had carcinomas, 7 had adenomas and in 4 cases the diagnosis was doubtful. Gynecomastia and testicular atrophy are the presenting clinical features. Increased amounts of estrogens are excreted. Other steroid findings vary. All possible causes of gynecomastia must be considered in the differential diagnosis (p. 487). As with other tumors of the adrenal cortex (p. 351), radiological demonstration of the tumor is of great importance.

G. Assessment of Adrenocortical Function

J. MÜLLER

For the exact methodology required for the determination of individual adrenocortical hormones and their metabolites and of the peptide and protein hormones which regulate their secretion, the reader is referred to review articles and original publications. In this section only the principles and normal values of hormone assays which are of diagnostic importance – mainly the practically important group determinations – are mentioned, but exact procedures for adrenal function tests will be described.

1. Determination of Plasma ACTH

The most sensitive *biological methods* for determination of ACTH are based on the bioassay of LIPSCOMB and NELSON (1962), and can reliably detect concentrations of 0.1 mU/100 ml (1 ng/100 ml) and more. In the method according to NEY (1963), the ACTH of 100 ml of blood is concentrated 20-fold with a carboxyl exchange resin. The extract is administered to hypophysectomized rats by i.v. injection, and corticosterone is determined fluorimetrically in blood taken from the left adrenal vein. Suppression by dexamethasone instead of hypophysectomy considerably decreases the sensitivity of the assay.

Normal values

Morning:	0.1–0.5 mU/100 ml;
Evening:	0.1–0.15 mU/100 ml;
Stress (surgery):	0.5–2 mU/100 ml.

Elevated values are found in untreated Addison's disease, Cushing's syndrome of pituitary-hypothalamic origin after adrenalectomy and substitution therapy, ACTH-secreting carcinoma, and the congenital adrenogenital syndrome (see Fig. 8, p. 298).

Minute amounts of ACTH can also be detected by radioimmunology. Guinea pigs produce antibodies against porcine ACTH, which cross-react with human ACTH. A mixture of plasma with unknown ACTH content, ACTH anti-serum, and ACTH labeled with ^{131}I of high specific activity is separated by electrophoresis. The concentration of ACTH in the plasma can be calculated from the ratio of free ^{131}I-labeled ACTH to antibody-bound ^{131}I-labeled ACTH.

Normal values

Morning: 0.4–0.9 mU/100 ml (YALOW, 1964);
0.3–0.7 mU/100 ml (DEMURA, 1966).

At present, both biological and radioimmunological ACTH assays are technically very difficult and are thus unsuitable for general diagnostic purposes.

2. Determination of Plasma MSH

MSH is estimated *biologically in vitro* or *in vivo*, with frog's skin by assessing an increase in pigmentation by eye, by microscopy, or by reflex spectrophotometry. Without chromatographic separation this method cannot, however, differentiate plasma activity due to α-MSH from that due to β-MSH or ACTH. Elevated values of total MSH activity are found in Addison's disease and in advanced pregnancy; decreased values in pituitary insufficiency.

Radioimmunological methods have been developed for the determination of both forms of MSH (ABE, 1967a and b). Up to now, however, only β-MSH has been unequivocally demonstrated in peripheral human plasma. α-MSH, can be found in extracts of human pituitary gland and of ectopic ACTH-producing tumors.

β-MSH

Normal values:	9 ng/100 ml;
Elevated values*:	50–600 ng/100 ml.

3. Plasma Renin and Angiotensin Assays

Since the concentrations of renin and angiotensin II in the blood are dependent, among other factors, on the effective plasma volume, estima-

* Addison's disease; Cushing's syndrome of pituitary-hypothalamic origin after bilateral adrenalectomy; ectopic ACTH-producing tumor.

tions must be carried out in subjects on a normal salt intake (6 to 10 g daily) in the fasting state and after 12 hours in a recumbent posture.

a) Bioassay of Plasma Renin

Most methods currently in use for the estimation of human plasma renin are based on one of the two following methods:

BOUCHER (1964) described a method in which plasma is incubated at 37 °C with no additional substrate. The angiotensin II formed from endogenous substrate during incubation is extracted with Dowex-50 and eluted. Its blood pressure-increasing activity is measured in a nephrectomized anesthesized rat. *Renin activity* is expressed in ng of angiotensin II per 100 ml of plasma.

Normal values

0–570 ng/100 ml (VEYRAT, 1964).

In the method used by BROWN (1964), renin is extracted from plasma by adsorption onto DEAE cellulose. The purified renin is incubated for 30 min to 96 hours with a standard ox-serum substrate. The unknown renin activity is measured from the rate at which angiotensin is liberated from the substrate. Angiotensin is also estimated biologically by its blood pressure-increasing effect in rats. The *renin concentration* is expressed in renin units.

Normal values

2–19 renin units per liter.

b) Radioimmunological Determination of Plasma Angiotensin II

In 1967, three different laboratories succeeded in developing sensitive and specific radioimmunological methods for the estimation of angiotensin II in plasma (VALLOTTON, BOYD, CATT). These methods appear to be better suited than bioassays to the determination of angiotensin and renin for purposes of clinical diagnoses.

Normal values

Plasma >0.8–5.6 ng/100 ml (BOYD);
Blood 2.1 ± 1.4 ng/100 ml (CATT).

c) Plasma Renin Assay by Radioimmunological Determination of Angiotensin I

The determination of angiotensin I has the advantage that the addition of converting enzyme and the difficult inhibition of the angiotensinases can be avoided and that antibodies to angiotensin I show less cross reactivity towards peptide fragments (HABER, 1969).

Normal values:

Plasma: intake of 110 mEq Na/d:
1.02–1.75 ng angiotensin I/ml/h
intake of 10 mEq Na/d:
2.16–5.92 ng angiotensin I/ml/h

4. Determination of Steroid Hormones

a) General Methodology

Clear identification and quantitative measurement of all the known steroid hormones and their most important metabolites in plasma and urine are now theoretically possible, but such analyses are technically very difficult, costly, and time-consuming, and they are only exceptionally used in clinical diagnosis. The relatively simple chemical group determinations of urinary and plasma steroids are usually adequate for the laboratory diagnosis of adrenal cortical function. Biological assay methods of steroid hormones are no longer used in clinical diagnosis.

Group determinations of steroids generally consist of the following steps: hydrolysis (when conjugated steroids are to be estimated), extraction with a suitable organic solvent, purification of the extracts, group-specific color or fluorescence reaction, and spectrophotometric or spectrofluorometric measurement. Most of these estimations can be carried out without difficulty in any reasonably large clinical chemical laboratory. When used in function tests they permit a reliable diagnosis of the most important adrenocortical diseases. On the other hand, a single steroid determination in urine or plasma only exceptionally allows definite exclusion or confirmation of a diagnosis of adrenocortical disorder. Because many drugs (e.g. psychotropic agents) can impair or distort chemical steroid estimations due to false color reactions, it is always advisable to discontinue all drugs not absolutely necessary for a few days before a steroid analysis.

Numerous *chromatographic methods* (adsorption or partition chromatography on columns, paper, thin-layer, or gas chromatography) permit perfect separation and identification of individual steroids in most cases. However, a single chromatography is hardly ever adequate for the isolation of a pure steroid fraction from biological material; this is only possible if chromatography is repeated in different solvent systems and often only if it is combined with chemical modifications of the steroid molecule. Losses which might cause quantitative inaccuracies can be avoided by *isotope methods*. In simple isotope dilution methods, a tracer dose of tritiated or ^{14}C-labeled steroid is added to

the plasma or urine sample at the beginning of the analysis in order to check losses of material occurring during purification. Quantitative determination of the steroid isolated and purified by chromatography can be performed by a colorimetric, fluorometric or gas chromatographic method or by competitive binding to a specific carrier protein or antibody (DICZFALUSY, 1970; MURPHY, 1969). In double isotope dilution derivative assays, the unknown steroid concentration is also quantitatively determined by a radiochemical method, e.g. esterification of the steroid with labeled acetic anhydride. These methods, which are qualitatively and quantitatively very reliable but technically demanding, are used in clinical diagnosis, e.g. for the determination of plasma testosterone or urinary aldosterone, where simpler methods such as are used for the determination of cortisol and its metabolites are not available. Radioimmunoassays have been developed for the determination of steroid hormones, such as testosterone, aldosterone, corticosterone, deoxycorticosterone, and progesterone. These methods are somewhat simpler than double isotope dilution derivative assays, but generally still require at least one chromatography and the use of an internal radioactive standard for checking procedural losses.

Methods of estimating *steroid secretion rates or production rates* are also based on the principle of isotope dilution; the mixing between unlabeled and radioactive-labeled steroid occurs within the organism.

b) Plasma Steroid Determinations

α) Fluorometric Determination of Plasma Corticosteroids

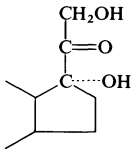
The two 11β-hydroxycorticosteroids cortisol and corticosterone can be determined by the fluorometric methods introduced by SWEAT (1952) and SILBER (1958). Since the fluorescence reaction due to corticosterone is three times more intense than that due to cortisol and the ratio of cortisol to corticosterone in the plasma is approximately 10 : 1, values obtained are 30% higher than when the Porter-Silber method (see below) is used, which measures almost exclusively cortisol. Moreover, every plasma extract produces a minor nonspecific fluorescence reaction. Single values must thus be carefully interpreted, because there is no definite limit between normal and decreased values. However, the method is very useful for repeated determinations in function tests. It is considerably more sensitive than the Porter-Silber reaction and yields precise and reproducible results even when the plasma cortisol concentration is in the normal or lower range. The technical procedure is very simple and results are available within a short time. We use a method described by SILBER (1966). Similar methods which are frequently used have been described by DE MOOR (1960) and MATTINGLY (1962).

Normal values

10–30 μg/100 ml depending on the time of day;
false high values during spironolactone medication.

β) Plasma 17-Hydroxycorticosteroids (Porter-Silber Chromogens)

The 17α,21-dihydroxy-20-ketone side chain of cortisol and its most important metabolites



react with phenylhydrazine in the presence of alcohol and sulfuric acid to produce a yellow color which can be measured by spectrophotometry. This so-called Porter-Silber reaction is used in the method of PETERSON for the relatively specific determination of cortisol (free cortisol and protein-bound cortisol, but not conjugated cortisol metabolites) in 5 ml of heparinized plasma. The method can be used for the metopyrapone test, because in contrast to the fluorometric methods it also measures cortexolone (compound S). It does, however, have the disadvantage that low normal plasma cortisol concentrations are at the lower limit of sensitivity of the Porter-Silber reaction.

Normal values

6–25 μg/100 ml, depending on the time of day;
false high values: ketone bodies, quinine, psychotropic drugs.

γ) Plasma Aldosterone

By using a modification of the original double-isotope method developed by KLIMAN (1960) for the determination of aldosterone in urine and in adrenal venous blood, PETERSON (1964) succeeded in measuring aldosterone in 20 to 30 ml of peripheral plasma. However, this method involved the use of ^{14}C-labeled acetic anhydride with a high specific activity and was too costly for purposes of clinical diagnosis. More recently, two similar methods have been developed in which aldosterone is esterified

with tritiated acetic anhydride (BRODIE, 1967; COGHLAN, 1967). Although these methods are also technically difficult and time-consuming, they are at least financially acceptable. They may later be replaced by radioimmunoassays.

Normal values

2–15 ng/100 ml;
primary aldosteronism:
23–88 ng/100 ml (during normal sodium intake).

δ) Plasma Androgens

Estimation of the plasma 17-ketosteroids is of no clinical importance. They consist mainly of the biologically inactive sulfates of dehydroepiandrosterone and androsterone. The determination of single biologically active androgens in the plasma is of greater significance and can be carried out in small amounts of plasma by means of a double isotope dilution derivative method (RIONDEL, 1963; HUDSON, 1963), a simple isotope-dilution method with gas chromatography with a highly sensitive electron-capture detector (BROWNIE, 1964), competitive binding to a carrier protein, or radioimmunoassay.

Normal values (according to GANDY, 1965)

Testosterone
Men: 0.38–1.19 μg/100 ml;
Women: 0.01–0.08 μg/100 ml.
Androstendione
Men: 0.05–0.06 μg/100 ml;
Women: 0.03–0.33 μg/100 ml.
Dehydroepiandrosterone
Men: 0.13–1.4 μg/100 ml;
Women: 0.14–1.06 μg/100 ml.

c) Determination of Urinary Steroids

Determinations of single steroids or a group of steroids in the 24-hour urine are frequently used in clinical diagnosis. The daily secretion rate of steroid hormones can be estimated from the excretion of representative urinary metabolites, which is not possible from the determination of plasma steroids. Group determinations of urinary steroids are also frequently used for stimulation and suppression tests.

The most common source of error in the determination of urinary steroids is the incorrect collection of a 24-hour urine specimen. In many cases it is advisable to monitor the daily urine output by means of the relatively constant creatinine excretion. The urine is best stored without the addition of a preservative under refrigeration at 2 to 4 °C for a few days at the most. When the analysis has to be postponed for a longer period, the urine must be frozen and stored in the deep freeze.

α) Urinary 17-Hydroxycorticosteroids (Porter-Silber Chromogens)

The urinary 17-hydroxycorticosteroids (17-OH-CS), which we determine by the reliable method of PETERSON (PETERSON, 1955; FIEDLER-BEHRENDT, 1962), account for 30 to 40% of the cortisol secreted in 24 hours and include all compounds with an intact 17,21-dihydroxy-20-ketone side chain. Metabolites of corticosterone and aldosterone are not determined, but tetrahydro-S, the most important metabolite of cortexolone, is. 20 ml of urine is incubated with β-glucuronidase at 37 °C for 24 hours and extracted with methylene chloride. The extract is washed with sodium hydroxide and treated with the phenylhydrazine reagent of Porter-Silber. This leads to the formation of a yellow pigment which can be measured spectrophotometrically. The unknown steroid concentration can be calculated from the optic density at 410 nm after subtraction of a sulfuric acid blank of the urine extract.

Normal values

3–13 mg/24 h, or
1.7–7.2 mg/m^2 for children.

Low Values. Low values are characteristic in Addison's disease and hypopituitarism but are not adequate for diagnosis of these conditions. A low excretion rate is also found in cirrhosis of the liver, myxedema, and old age.

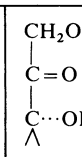
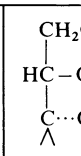
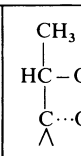
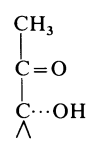
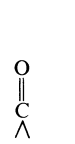
Elevated Values. Elevation of the 17-OH-CS in the urine is characteristic in Cushing's syndrome, but may also be due to thyrotoxicosis or fever. The 17-OH-CS excretion is not proportional to the body weight, but in obesity a moderate increase in 17-OH-CS excretion up to 18 mg per day is often observed (see p. 354). In equivocal cases, obesity can best be differentiated from Cushing's syndrome by a dexamethasone suppression test (2 mg per day; see p. 389).

False High Values. Ketosis; quinine, meprobamate, phenothiazines (and other psychotropic drugs), and antiepileptic agents.

β) 17-Ketogenic Steroids According to NORYMBERSKI and "Total 17-Hydroxycorticoids" According to APPLEBY

The C_{21}-steroids which are hydroxylated in position 17 can be converted to 17-ketosteroids

Table 18. Color reactions and reactive groups of the side chain used in the determination of urinary steroids

Side chain	 Dihydroxy-acetone	 Glycerol	 17-20-glycol	 17-20-ketol	 17-ketone
Examples	Cortisol Cortisone TH-cortisol TH-cortisone Cortexolone TH-S	Cortol Cortolone	Pregnanetriol	17α-OH-pro-gesterone	Dehydroepiandrosterone Androstenedione Androsterone
Porter-Silber reaction (17-OH-CS)	+	−	−	−	−
17-ketogenic steroids according to NORYMBERSKI	+	+	+	−	−
17-ketogenic steroids according to APPLEBY	+	+	+	+	−
Zimmermann reaction (17-ketosteroids)	−	−	−	−	+

(17-KS) by oxidation with sodium or potassium bismuthate and then measured by Zimmermann's reaction. In this method, enzymatic hydrolysis is not necessary because the 17-KS are relatively stable compounds which can be hydrolyzed by heating with acid. In Norymberski's procedure, the 17-ketogenic steroids (17-KGS) are calculated from the difference in the 17-KS measured before and after oxidation. Recently, sodium periodate has been used in place of sodium bismuthate for the oxidation.

According to Appleby's modification, the steroids are first reduced with sodium borohydride. This eliminates the 17-ketosteroids and allows the determination of the 17-KGS by only one colorimetric reaction. The simultaneous reduction of the 20-keto group to 20-hydroxy groups also causes the steroids 17-hydroxyprogesterone and 17-hydroxypregnenolone to become ketogenic (see Table 18). The term "total 17-hydroxycorticoids" has become accepted in England and Sweden for 17-KGS as estimated by Appleby's method; this can lead to confusion with the Porter-Silber chromogens.

The 17-KGS include more cortisol metabolites than the Porter-Silber chromogens, since cortol and cortolone are estimated in addition to the tetrahydro derivatives. On the other hand, steroids which are not derived from cortisol, such as pregnanetriol are also estimated. Thus, there are advantages and disadvantages to the assessment of cortisol production as against the Porter-Silber chromogens.

Normal values according to BORTH (1957) (total 17-hydroxycorticoids)

Men: 6–21 mg/24 h;
Women: 4.5–16 mg/24 h.

γ) Free Urinary Cortisol and Corticosteroids

Determination of free, unmetabolized and unconjugated urinary cortisol is a valuable index of the concentration of free, non-protein-bound cortisol in the plasma providing renal function is normal (see p. 292). In contrast to the urinary 17-hydroxycorticoids, free urinary cortisol is only indirectly dependent on the cortisol secretion rate and metabolic clearance by the liver. It actually represents the amount of cortisol which has been at the disposal of the tissue cells during one day and it correlates with the functional state of the adrenal cortex more closely than any other parameter. The determination is not simple, however, and can therefore only be employed for clinical diagnosis in special cases (BROOKS, 1963; ROSNER, 1963).

Normal values

< 200 μg/24 h.

Substantial elevations are observed in Cushing's syndrome.

Direct fluorometric estimation of "cortisol" in urine extracts, in analogy to the estimation of plasma corticoids, leads to average values 3 times higher than those obtained by quantitatively and qualitatively exact analysis with isotope dilution and paper chromatography (ESPINER, 1965). Because the cortisol fraction of the total fluorogenic free urinary corticoids varies between 10 and 80% a determination of this kind cannot replace an exact analysis of free urinary cortisol. Nevertheless, MATTINGLY (1964) found very good correlation between the results of a simple assay like this and cortisol secretion rates as measured by isotope dilution in normal and ACTH-treated subjects, as well as in patients with Cushing's syndrome. The simple and quick fluorometric assay of free urinary corticoids can therefore partially replace the technically more difficult and time-consuming determinations of 17-hydroxycorticoids or 17-ketogenic steroids.

Normal values

78–372 μg/24 h (MATTINGLY, 1964).

δ) Urinary Aldosterone Metabolites. Determination of Aldosterone Secretion Rate

Since the introduction of the double-isotope derivative assay of urinary aldosterone-18-glucuronide by KLIMAN and PETERSON (1960), the previously used method of NEHER and WETTSTEIN (1956) has been abandoned. Various modifications have also been made to the double-isotope method. In our laboratory, we have found the procedure described by NEW (1966) to be very reliable. The principle of the method is as follows: A tracer dose of ^{14}C-labeled aldosterone is added to a urine sample. For hydrolysis of aldosterone-18-glucuronide the urine is acidified to pH 1 and kept at room temperature for 24 hours. The aldosterone liberated is extracted with methylene chloride and a preliminary purification by solvent partition and paper chromatography is performed. Esterification with tritium-labeled acetic anhydride leads to the formation of aldosterone-^{3}H-diacetate. This is followed by paper chromatography in three different solvent systems. Tritium and ^{14}C activities are measured in the purified samples by liquid scintillation counting. The amount of aldosterone in the counting vial can be calculated from the tritium activity and the known specific activity of the ^{3}H-acetic anhydride. The ^{14}C activity indicates which fraction of the original amount of aldosterone in the urine sample is still present in the counting vial and thus allows correction of any procedural losses.

Aldosterone secretion is dependent on sodium and potassium balance. Therefore, a patient in whom an aldosterone analysis is to be performed must be maintained on a liberal sodium (at least 100 mEq per day = 6 g of salt) and fluid intake; diuretics and potassium tablets must be discontinued for a few days.

Normal values

Adults: 5–20 μg/24 h;
Children: 3–13 μg/24 h per m^2 of body surface.

Because in liver diseases and pregnancy a relatively high proportion of secreted aldosterone is excreted as aldosterone-18-glucuronide, in certain cases it is also advisable to measure urinary tetrahydroaldosterone-3-glucuronide. A double-isotope procedure for measuring this metabolite has been described by NEW (1966).

Determination of the aldosterone secretion rate permits exact assessment of aldosterone production. Tritium-labeled aldosterone is injected intravenously and the specific activity of urinary aldosterone-18-glucuronide is determined by a method analogous to that of KLIMAN and PETERSON (1960) (using ^{14}C-acetic anhydride). The specific activity of urinary tetrahydroaldosterone-glucuronide can also be measured (ULICK, 1958). Because aldosterone-18-glucuronide is excreted more quickly than tetrahydroaldosterone-3-glucuronide (SIEGENTHALER, 1964), the collection of a 24-hour urine sample is sufficient for the first method, whereas a 48-hour urine sample has to be collected when tetrahydroaldosterone-glucuronide is measured.

Normal values

Adults: 50–200 μg/24 h.

ε) Urinary 17-Ketosteroids

We have found the procedure of PETERSON and PIERCE (1960) very reliable for the classic estimation of urinary 17-ketosteroids by Zimmermann's reaction. The 17-ketosteroids (17-KS) include the metabolites of adrenal and testicular androgens, which are inactive or only slightly active. Cortisol metabolites account for only a low proportion. Major 17-ketosteroids are androsterone, etiocholanolone, their 11-keto- and 11-hydroxy derivatives, and dehydroepiandrosterone. Although androsterone is the most important metabolite of testosterone, neither total 17-ketosteroid nor androsterone excretion can be considered as being representative of testosterone secretion. In normal conditions, 60% of the 17-KS in the male and

almost all the 17-KS in the female originates from the adrenal cortex. Practically all the urinary 17-KS are conjugated with sulfuric acid or glucuronic acid.

A 5 ml sample of the 24-hour urine is hydrolyzed by boiling with acid, and is then extracted with a mixture of petroleum ether and benzene; the extract is purified by washing with alkali. The 17-keto group (see Table 18) produces a purple color with *m*-dinitrobenzene in the presence of alkali (Zimmermann's reaction). This purple derivative can then be extracted with methylene chloride and measured spectrophotometrically.

Normal values are dependent on sex and age (Fig. 36, BORTH, 1957, see also Chap. XIX)

Adults

Men:	10–25 mg/24 h;
Women:	5–15 mg/24 h.

Children

First days of life:	up to 1.5 mg/24 h;
First year:	less than 0.5 mg/24 h;
2nd to 5th years:	less than 1 mg/24 h;
6 th to 9th years:	less than 2 mg/24 h;
10th to 15th years:	less than 10 mg/24 h.

Decreased Values. Addison's disease, pituitary insufficiency, cirrhosis of the liver, cachexia, advanced age.

Elevated Values. Very high values of up to more than 100 mg/24 h are found in adrenocortical carcinoma; high values in congenital adrenogenital syndrome, in the presence of Leydig cell tumors and virilizing adrenal cortical adenomas; elevated or normal values in Stein-Leventhal syndrome, arrhenoblastoma and simple hirsutism. A moderate increase is usually but not always found in Cushing's syndrome. Very high values in Cushing's syndrome are suggestive of an adrenocortical carcinoma.

ζ) Determination of Individual Urinary Androgen Metabolites. Androgen Production Rates

The numerous procedures developed for the fractionation and chromatography of urinary 17-ketosteroids have provided valuable information on human androgen metabolism but are of little importance in clinical diagnosis. No definite conclusions about the gonadal or adrenal origin of 17-ketosteroids or the malignancy of an adrenal tumor are possible from the percentage of individual 17-ketosteroid fractions.

Although dehydroepiandrosterone, 11-hydroxy- and 11-keto-17-KS are derived mainly from the adrenals, they can also be produced by a mixed embryonic tumor of the testis. The same enzyme defect may be present in congenital adrenogenital syndrome, as well as in malignant adrenocortical tumors. In contrast to an earlier opinion, there are probably no specific adrenal cortical androgens leading to hirsutism and not to virilization. Elevated plasma levels of testosterone in women with simple hirsutism rather indicate that the hirsutogenic activity of individual androgens parallels their virilizing activity.

In contrast to the 17-KS, urinary testosterone-glucuronide is only derived from testosterone. The determination of this metabolite in 24-hour urine allows certain conclusions about the endocrine function of the testis in man. However, only 0.15 to 1.9% of the testosterone secreted is excreted as testosterone-glucuronide in the urine (CAMACHO, 1964). The determination is of a similar degree of technical

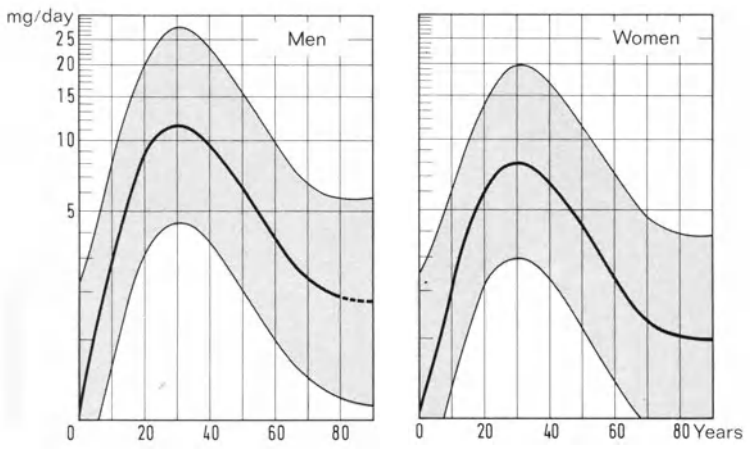


Fig. 36. Mean values of urinary 17-ketosteroids for normal individuals with average body weight and average length. Mean = solid line; limits of confidence = stippled area (P = 0.05). (From BORTH, LINDER, and RIONDEL, 1957)

difficulty to the measurement of plasma testosterone (see p. 381).

Normal values

Men: 15–520 μg/24 h (DULMANIS, 1964).

In women, most of the urinary testosterone-glucuronide is derived from androstenedione, which is secreted by the adrenal cortex and converted to testosterone by the liver. Since most of the testosterone is immediately conjugated by the liver and secreted into the blood stream as the glucuronide, the determination of urinary testosterone-glucuronide in women does not permit valid conclusions about the amount of testosterone secreted by endocrine glands or the amount of testosterone secreted into the blood stream during one day. This leads to wide differences between "blood production rates" and "urine production rates" of testosterone in women. The blood production rate is calculated by multiplication of the plasma testosterone concentration with the metabolic clearance rate. The urine production rate is calculated from the specific activity of testosterone-glucuronide in the 24-hour urine after intravenous administration of radiolabeled testosterone.

In men, determination of the testosterone urine production rate permits valid conclusions about the secretion of this hormone by the testes.

Normal values

Men: 4–12 mg/24 h (KORENMAN, 1963).

5. Stimulation Tests

a) ACTH Tests (Thorn Test) for the Assessment of Adrenal Cortical Function and Functional Reserve

The ACTH test, i.e. the determination of urinary or plasma steroids before and after stimulation of the adrenals with an ACTH preparation, is the most important test of adrenocortical function and is essential for the diagnosis of adrenal insufficiency.

There are numerous modifications of the ACTH test. ACTH may be given intravenously, either by injection or by infusion, or intramuscularly; determinations of various steroid fractions in plasma and urine and blood eosinophil counts can be performed at different times. For a review of the many original procedures with their normal values see PRUNTY. However, new modifications of this test are recommended almost every week. A critical evaluation of all these variations of the test is impossible. We can deal only with a few basic principles and a few modifications of the tests which we have found most reliable in 20 years of experience.

In agreement with RENOLD (1952), we have observed maximal stimulation of the adrenal cortex with a dose of 25 IU of a water-soluble natural or synthetic (0.25 mg) ACTH preparation administered by intravenous infusion over 8 hours. In all persons with a normal adrenocortical function, this causes the plasma corticoids as measured by fluorometry to rise to a value at least twice as high, in some instances three or four times as high, as the initial value. The 24-hour value of urinary 17-hydroxycorticoids as measured by the Porter-Silber reaction after enzymatic hydrolysis (see p. 381) also increased to 2 or 3 times the baseline value in all normal subjects on the day such an ACTH infusion was given. Intramuscular administration of 120 IU of a natural or 1 mg of a synthetic long-acting ACTH preparation leads to a similar optimal stimulation, which is characterized, however, by a later onset and a longer duration. Failure of the urinary or plasma corticoids to respond to ACTH in this dosage definitely indicates adrenal insufficiency. When the test is repeated on two or three consecutive days with no response, the diagnosis of Addison's disease is confirmed.

The assessment of an ACTH test by the fall in blood eosinophils is only permissible in emergencies or as an additional check. Only a positive result indicates an intact adrenal function, whereas false negative results are obtained in 30% of all tests. When a reliable assay for plasma or urinary corticoids is available, a simultaneous eosinophil count is pointless since it makes it impossible to carry out the ACTH test under steroid cover (see below). Determination of the plasma corticoids by a fluorometric method is no more difficult or time-consuming than an eosinophil count.

The response of urinary 17-ketosteroids to ACTH is less distinct and is often delayed by 24 hours. When a reliable assay of urinary 17-hydroxycorticoids or 17-ketogenic steroids is available, simultaneous estimation of the 17-ketosteroids is not necessary.

The ACTH test is also often used in the differential diagnosis of adrenal hyperfunction. In Cushing's syndrome with bilateral adrenal hyperplasia, there is a pronounced or even excessive rise in the urinary and plasma corticoids, whereas in cases of adrenal tumors the response is reduced or absent. The ACTH test does not, however, permit definite differentiation between Cushing's syndrome and normal adrenal function. It is therefore not advisable to perform an ACTH test to differentiate between

hyperplasia and a tumor until a diagnosis of Cushing's syndrome has been established by a low-dose dexamethasone suppression test.

Although different authors have suggested that the ACTH test also be subjected to quantitative evaluation (JENKINS, 1955; BIRKE, 1958) and that partial or relative adrenocortical insufficiency can be deduced from a less pronounced rise in corticosteroids, we think the result can only be positive or negative, showing whether the adrenals are responsive or not. In doubtful cases we repeat the test.

Side effects of ACTH are uncommon. Hypersensitivy reactions, however, can occur particularly in adrenal insufficiency and after repeated administration of ACTH. Immediate reactions of the anaphylactic type are pruritus, urticaria, exanthema, or asthma, which respond to antihistamines. A delayed reaction may occur a few hours after an ACTH infusion is started in the form of fever and vomiting and can be very dangerous in adrenocortical insufficiency. It is therefore advisable to perform all ACTH tests under cover of dexamethasone in a dosage of 0.5 mg p.o. twice daily. This does not interfere with the steroid analysis used for assessing the adrenocortical response to ACTH, and undesired side effects are avoided. Interpretation of the reduced eosinophil count is not possible during corticosteroid medication.

α) Eosinophil Count

Capillary or venous blood is used for the eosinophil count; calculation from the percentages obtained in blood smears is not sufficiently precise. The direct eosinophil count according to RANDOLPH is very reliable. Either capillary blood from the finger tip or venous blood can be used. In the latter case, 5 ml of blood are taken from the cubital vein and mixed at once with a weighed amount of an oxalate mixture. The oxalate-and-blood mixture can be stored in the refrigerator for up to 6 hours.

The composition of the oxalate mixture is: Ammonium oxalate 1.2 g, potassium oxalate 1.8 g, distilled water to give 100 ml. A pipette is used to transfer 0.5 ml of this mixture to each tube to be used for blood collection, where it is evaporated.

Randolph's solution has proved successful for selective staining of the eosinophils: phloxine 0.1, propylene glycol 50 ml, distilled water 50 ml. The solution can be kept indefinitely.

Capillary or venous blood is drawn up into a leukocyte pipette to the 0.5 mark, and then diluted with Randolph's solution up to the 11 mark. The pipette can then be left for up to 12 hours. It must be vigorously shaken for at least 2 min, and 3 to 5 min must elapse between filling the counting chamber and counting. The eosinophils are counted in 2 or preferably 4 Fuchs-Rosenthal cerebrospinal fluid counting chambers 0.2 mm in depth. The following formula is used for the calculation:

$$\text{Eosinophils per mm}^3 = \frac{\text{eosinophils per chamber} \times 100}{16} = \text{eosinophils per chamber} \times 6.25.$$

The staining method of DUNGER is useful when many estimations have to be performed, but the determination must be completed at once. Other methods of selective staining have been described by HENNEMANN and HINKELMANN.

β) Rapid ACTH Test (8-Hour Intramuscular Long-Acting ACTH Test)

The plasma corticoids are measured fluorometrically or colorimetrically (Porter-Silber reaction), or the blood eosinophils are counted before and 8 hours after an i.m. injection of long-acting ACTH (ACTH-Zn). Alternatively, the 24-hour urinary output of 17-hydroxycorticoids or 17-ketogenic steroids is determined on the day before the test and on the day the injection of ACTH is given. A blood sample is taken at 8 a.m., after which 120 IU of a natural or 1 mg of a synthetic long-acting ACTH preparation is given by i.m. injection to adults. The dose for children is 75 IU or 0.6 mg per m^2. A second blood sample is taken at 4 p.m.

When the plasma or urinary corticoids increase by 100% or more, or when the eosinophils decrease by 50% with a base-line value of more than 100 per mm^3, primary adrenocortical insufficiency can be definitely excluded. However, a negative result of the eosinophil test is no proof of adrenocortical insufficiency. When the result of a rapid ACTH test is negative, a prolonged ACTH test (with long-acting ACTH or repeated intravenous ACTH infusions) should be performed.

The rapid ACTH test is very suitable for ambulant patients.

γ) Prolonged ACTH Test

An i.m. injection of 120 IU, or 1 mg of a long-acting ACTH preparation, is given once or twice (with an interval of 8 to 12 hours) daily, depending on the duration of action of the preparation used. In patients with secondary adrenocortical insufficiency, a distinct increase

of urinary or plasma corticosteroids can be observed on the second or third day of ACTH treatment in most instances. However, an unequivocal increase may not be seen until after a week of treatment.

δ) 8-Hour Intravenous ACTH Test

ACTH given by intravenous infusion stimulates the adrenal cortex to the greatest possible extent and is therefore the most sensitive test for assessment of its functional reserve. It makes it possible to evaluate not only the immediate secretory increase (actual functional reserve) but, when administered for several days, also the maximum hormone production possible after development of adrenocortical hyperplasia, i.e. the "potential functional reserve" according to RENOLD (1952). An additional advantage is that an ACTH infusion can be interrupted at any time if a patient shows signs of a hypersensitivity reaction.

As a standard procedure, intravenous administration of ACTH over 8 hours on two consecutive days has proved very reliable. On the first day of the test the 24-hour urinary output of 17-hydroxycorticoids and 17-ketosteroids is measured (base-line). On the second day a blood sample is taken at 8 a.m. in the fasting state for the measurement of plasma corticoids and the eosinophil count. This is immediately followed by an intravenous infusion of 25 IU or 0.25 mg (children 12 IU or 0.12 mg per m^2) of ACTH in 500 ml (300 ml per m^2) of physiological saline solution over 8 hours. A second blood sample is taken at 4 p.m. Urine is collected from 8 a.m. on the second day to 8 a.m. on the third day of the test for determination of 17-hydroxycorticoids. The same procedure is followed on the third day of the test.

A fall of more than 85% (average 94%) in the blood eosinophils, an increase of the plasma corticoids to at least 40 μg/100 ml and to 100% above the base-line value, or an increase of 10 to 15 mg per 24 hours or 100% in the urinary 17-hydroxycorticoids indicates normal adrenocortical function and definitely excludes Addison's disease. An inadequate fall in eosinophils and failure of the corticosteroids to rise indicate adrenal insufficiency. In normal subjects, the rise in plasma corticoids and the fall in the eosinophils in response to the first and the second ACTH infusion are the same. In secondary adrenocortical insufficiency the ACTH effects is more pronounced on the third day of the test. In contrast, the urinary corticoids increase further on the third day of the test in normal subjects also. In the normal subject, the increase above the base-line value is between 4.5 and 30 mg per 24 hours on the second day of the test and between 5 and 41 mg per 24 hours on the third. In Cushing's syndrome with bilateral adrenal hyperplasia the increase of the 17-hydroxycorticoids can be as high as 50 to 80 mg per day. When Cushing's syndrome is due to an adrenal cortical adenoma, the response is variable. A definite increase of corticosteroid output is not common in adrenocortical carcinoma.

Cortisone-resistant eosinophilia can be recognized by the failure to respond to exogenous cortisone. The blood eosinophils are counted before and after the oral administration of cortisone acetate (adults 100 mg, children 60 mg per m^2). Alternatively, the eosinophils can be counted before and three hours after an intravenous infusion of cortisol hemisuccinate in a dose of 20 mg in 100 ml of physiological saline for adults or 12 mg per m^2 in 60 ml per m^2 for children, given over one hour. Constitutional aneosinophilia is not related to any adrenocortical disorder.

ε) Repeated Intravenous ACTH Test

A repeated intravenous ACTH test can be used in the same way as the prolonged intramuscular ACTH test for the differentiation between primary and secondary adrenocortical insufficiency. Secondary adrenal atrophy can be relieved by repeated intravenous stimulation in most instances, whereas primarily atrophic or damaged adrenals fail to react. A fall in the eosinophils can be examined on successive days as the eosinophils return to their original level overnight after an intravenous ACTH infusion.

b) Methopyrapone Test (Metopirone Test)

Principle. The adrenostatic drug methopyrapone inhibits the 11β-hydroxylase of the adrenal cortex and therefore blocks the synthesis of cortisol, corticosterone, and aldosterone without impairing production of their precursors cortexolone (compound S) and deoxycorticosterone. The failure of the negative feedback effect of cortisol on the hypothalamus results in a maximum output of ACTH, which elicits an increase in plasma and urinary 17-hydroxycorticoids and 17-ketogenic steroids corresponding to an increased formation of cortexolone (Fig. 37), providing the adrenal cortex responds normally to ACTH. When the adrenal glands are normal, the methopyrapone test allows assessment of the pituitary ACTH reserve.

Method. The test should be only carried out when the urinary 17-hydroxycorticoids are normal or have shown a normal response to ACTH. Any type of corticosteroid therapy must be discontinued during the test.

Day 1: determination of urinary 17-hydroxycorticoids (base-line value);
Days 2 and 3: 750 mg (or 30 mg per kg body weight) of methopyrapone are given orally every four hours after meals or with a glass of milk;
Day 4: no medication but collection of a 24-hour urine sample.

Urinary excretion of 17-hydroxycorticoids is measured on days 1, 3, and 4.

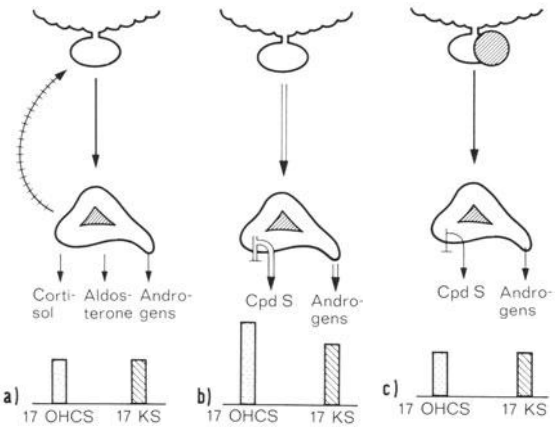


Fig. 37a–c. Methopyrapone test. a) Normal person before methopyrapone. b) Normal person under treatment with methopyrapone: suppression of 11-hydroxylase, production of cortisol blocked, instead cortexolone (compound S) is produced, which does not suppress ACTH secretion: increase in urinary corticosteroids. c) Patient with partial pituitary insufficiency treated with methopyrapone: cortexolone is secreted in place of cortisol. No increase in urinary corticoids because ACTH reserve is decreased

Interpretation. When the hypothalamic-pituitary-adrenocortical system is functionally intact, urinary 17-hydroxycorticoids increase by 100% or 10 mg per 24 hours above the base-line value on day 3 or 4 of the test. When the urinary 17-hydroxycorticoids respond to ACTH but not at all to methopyrapone a disturbance of ACTH secretion must be assumed. An inadequate rise with a normal base-line value is indicative of a limited ACTH reserve which can occur, for instance, after pituitary irradiation or in Cushing's syndrome due to an autonomous adrenocortical adenoma. The methopyrapone test is particularly suited for the assessment of a decreased ACTH reserve due to therapy with high doses of a cortisone derivative. However, cortisol secretion may respond normally to stress such as surgery in patients in whom the response to the methopyrapone test was pathologic, which can be explained by the different mechanisms of stimulation of ACTH secretion (see p. 299). Occasionally the test is also performed to differentiate between adrenocortical hyperplasia and tumor in CUSHING's syndrome. As a rule, the increase of 17-hydroxycorticoids is excessive in Cushing's syndrome with adrenocortical hyperplasia. In adrenocortical adenoma there is usually no increase even when the adenoma responds to ACTH.

We prefer to carry out the test under hospital conditions, although it can also be performed in out-patients. Regular intake of the drug every 4 hours even during the night is often not possible at home. Nausea and vomiting may occur during the test. These symptoms can be partially explained by a toxic effect of the drug, but they may also indicate adrenocortical failure. In patients with impaired ACTH secretion, there is the risk of an acute adrenocortical crisis provoked by a methopyrapone test.

In our opinion, the intravenous administration of methopyrapone (1 to 2 g or 30 mg/kg in 1 liter of physiological saline solution infused over 4 hours) offers no advantages.

c) Vasopressin Test

When pituitary insufficiency is suspected and the ACTH test is normal, an attempt can be made to stimulate the endogenous ACTH secretion by the administration of synthetic lysin-vasopressin. It has not been definitely established whether vasopressin acts directly on the pituitary gland or promotes the secretion of endogenous CRF (see p. 300). Its site of action seems to be below the center of negative feedback regulation and the site of action of stress. When a methopyrapone, insulin, or pyrogen test is pathologic, a normal vasopressin test indicates a lesion in the hypothalamus. The vasopressin test can also be used for the differentiation of Cushing's syndrome with adrenal hyperplasia from Cushing's syndrome due to an adrenal adenoma; no response in plasma cortisol can be observed in patients with an adrenal adenoma (BETHGE, 1968; TUCCI, 1968).

A blood sample is taken at 8 a.m., after which an intravenous infusion of 6 to 10 pressor units (depending on body weight) of synthetic lysin-8-vasopressin in 500 ml of physiological saline solution is given within two hours. A second blood sample for the determination of plasma corticosteroids is taken at 10 a.m. Normally the plasma corticoids increase by at

least 10 μg/100 ml or 100% above the base-line value.

Side effects are mild and consist of pallor, abdominal discomfort, and tenesmus.

d) Insulin and Pyrogen Stimulation Tests of the Pituitary Adrenal System

The nonspecific stimulation of the pituitary adrenal system by insulin-induced hypoglycemia or fever provoked by pyrogen can be used for diagnostic purposes. Only when the total hypothalamic-pituitary-adrenocortical axis is functionally intact can a rise in plasma corticosteroids be elicited by these measures. A definite increase of the plasma corticosteroid concentration in response to an intravenous injection of insulin can only be expected when a marked decrease in blood sugar is maintained for at least 10 minutes and leads to hypoglycemic symptoms. Whereas in most healthy individuals an insulin dose of 0.1 to 0.15 units per kg is sufficient, a higher dose (0.3 units per kg) is required to provoke a fall in blood sugar in patients with acromegaly. A maximum increase of the plasma corticosteroids by 13 μg/100 ml (5.4 to 18.4 μg/100 ml, LANDON, 1963) above the base-line value can be observed between 30 and 90 min after the insulin injection. Special caution is necessary in patients with pituitary insufficiency because a severe hypoglycemic coma may result.

In the pyrogen test according to ENGEL (1960), the plasma corticosteroids are determined in the early afternoon; pyrexal is then given in a dose of 0.2 μg by the i.v. route. A maximum increase of an average of 22.1 μg/100 ml ± 8.9 μg per 100 ml in the plasma corticosteroids is reached after three hours in normal subjects. In the test according to JENKINS (1964), a dose of 0.005 μg/kg body weight of pyrogen (Organon) is given by i.v. injection; in the healthy subject, the plasma corticosteroids increase by an average of 15 μg/100 ml within three hours. The test is terminated by an i.v. injection of 100 mg of cortisol hemisuccinate.

6. ACTH Suppression Tests with Dexamethasone

Principle. Small amounts of very potent glucocorticosteroids, such as dexamethasone, suppress ACTH secretion while contributing to the urinary or plasma concentrations of 17-hydroxycorticosteroids only minimally. These tests are clinically important

1. for the evaluation of ACTH-dependence or autonomy of adrenocortical steroid production, i.e. for the differentiation of normal and hyperplastic adrenals from adrenal tumors;

2. for a quantitative assessment of the suppressibility of ACTH secretion and thus for the differentiation between normal and uncontrolled pathologic function of the hypothalamic pituitary adrenal axis.

Whereas in most instances a determination of urinary or plasma corticoids before and during suppression is sufficient, the urinary 17-ketosteroids are also measured when adrenogenital syndrome or a cortisol- and androgen-producing tumor is suspected.

a) Overnight Dexamethasone Suppression Test

This test is an excellent screening procedure for Cushing's syndrome in outpatients. On the first day, a blood sample is taken at 8 a.m. for the determination of the base-line value of plasma corticosteroids (fluorometric or Porter-Silber method). A dose of 1 mg of dexamethasone is taken orally between 11 p.m. and midnight. A second blood sample is taken at 8 a.m. the following day. In normal subjects the concentration of the plasma corticoids decreases to less than 10 μg/100 ml or less than half the base-line value on the second day. In CUSHING'S syndrome of any etiology no response or an inadequate fall in plasma corticosteroids is observed.

The time of dexamethasone intake is very critical, since the morning rise of ACTH secretion cannot be blocked after midnight. We have found the test very reliable. Although a single dose of 1 mg of dexamethasone induces a significant fall in the plasma corticoids even in very obese patients, we increase the dose to 2 mg when the body weight exceeds 100 kg. Plasma corticoids did not decrease in any of more than a dozen patients with Cushing's syndrome, even when the base-line values were normal or only slightly elevated.

b) Low-Dose Dexamethasone Test (2 mg per day)

This reliable test, which was introduced by LIDDLE (1960), differentiates Cushing's syndrome of every etiology from simple obesity or any other disease with a high degree of accuracy. The original method entailed administration of 2 mg of dexamethasone on each of two successive days. We have found that there are certain advantages when dexamethasone is given on three successive days instead.

Method

Day 1: 24-hour urine collection for the determination of base-line steroid values;

Days 2 to 4: dexamethasone, 0.5 mg every 6 hours orally. Determination of urinary 17-hydroxycorticoids, perhaps also 17-ketosteroids, on days 3 and 4.

Interpretation. When the hypothalamic-pituitary-adrenal system is normal both urinary steroid fractions fall to values below 4 mg per 24 hours or less than half the base-line values, even when the base-line values of the 17-hydroxycorticoids (in obesity) or 17-ketosteroids (in simple hirsutism) are elevated. In Cushing's syndrome, the decrease is absent or inadequate. In rare cases of Cushing's syndrome with bilateral adrenal hyperplasia, administration of 2 mg of dexamethasone may provoke a paradoxical increase of the 17-hydroxycorticoids and 17-ketosteroids (BROWN, 1973). In the congenital adrenogenital syndrome, this low dose of dexamethasone is sufficient to suppress the ACTH secretion and normalize the elevated urinary 17-ketosteroids.

c) High-Dose Dexamethasone Test (8 mg per day)

When the diagnosis of Cushing's syndrome has been confirmed (by a pathologic low-dose dexamethasone suppression test), this test can differentiate adrenal hyperplasia from an adrenal tumor in most instances. It is less reliable than the low-dose dexamethasone test, however, and a number of cases of Cushing's syndrome with bilateral adrenal hyperplasia have been described in which the elevated urinary 17-hydroxycorticoids were not suppressed by 8 mg of dexamethasone (SILVERMAN, 1963; KATZ, 1966; LINN, 1967). The test can also be negative in nodular dysplasia (see p. 353).

Method

Day 1: base-line values of urinary 17-hydroxycorticoids and 17-ketosteroids;

Days 2 and 3: dexamethasone, 2 mg, every 6 hours orally. Determination of urinary 17-hydroxycorticoids and perhaps also 17-ketosteroids on day 3.

Interpretation. The inappropriate ACTH secretion of Cushing's syndrome of hypothalamic pituitary origin is suppressed by this dose and the steroid excretion decreases by more than 50%. In contrast, in cases of adrenocortical adenoma or carcinoma, the steroid excretion is not usually affected. Nor is the ectopic formation of ACTH in tumors, which leads to adrenal hyperplasia (paraneoplastic Cushing's syndrome), suppressed by 8 mg of dexamethasone, or only in rare instances (STROTT, 1968). A paradoxical increase of urinary 17-hydroxycorticoids during a high-dose dexamethasone suppression test has been observed in two individuals with apparently normal adrenocortical function, in whom the 17-hydroxycorticoids were normally suppressed by 2 mg of dexamethasone (ROSE, 1969). Diphenylhydantoin enhances hepatic conjugation of dexamethasone and may cause false positive 2 mg, but no 8 mg dexamethasone tests (JUBIZ, 1970).

7. Function Tests for Detection of the Source of Androgens in Hirsutism and Virilization

In the normal male, more than 90% of the plasma testosterone is secreted by the testes, whereas in the normal female more than half the plasma testosterone indirectly originates from the adrenal cortex. The major part is secreted as androstenedione by the adrenals and is converted to testosterone in the periphery (HORTON, 1966). When the plasma testosterone is elevated in a hirsute or virilized woman, an attempt can be made to detect the organ of origin by suppression and stimulation tests. Theoretically, it can be assumed that plasma testosterone deriving from the adrenals is stimulated by ACTH and suppressed by dexamethasone, whereas testosterone deriving from the ovaries is stimulated by chorionic gonadotropin and suppressed by estrogens, unless there is a completely autonomous hormone production due to a tumor. However, these assumptions have been only partially confirmed and valid procedures for androgen suppression and stimulation tests in the woman have not yet been established. Administration of ACTH did not stimulate the testosterone production in normal women, but had a stimulatory effect in a girl with congenital adrenogenital syndrome (KORENMAN, 1965). Prednisone significantly decreases plasma testosterone in normal women. Whereas in the normal male the plasma testosterone increases in response to treatment with chorionic gonadotropin (5000 units daily i.m. for five days) (LIPSETT, 1966), the same treatment led to a distinct rise in plasma testosterone in normal women only after pretreatment with human FSH (KORENMAN, 1965; LAMB, 1964). In contrast, the plasma testosterone rose in response to chorionic gonadotropin even without FSH pretreatment in women with the Stein-Leventhal syndrome (DIGNAM, 1964; KORENMAN, 1965). Whereas in the normal man the production and plasma concentration of testosterone can be significantly suppressed by treatment with a synthetic androgen such

as 2α-methyldihydrotestosterone-proprionate, 15 mg daily (DAVIS, 1965) or 9α-fluoro-11β-hydroxy-17α-methyltestosterone, 40 mg daily (LIPSETT, 1966) or an estrogen such as ethinyl-estradiol, 1 mg daily, corresponding results have not yet been found in the woman.

Because in most instances, virilization and endocrine hirsutism of the woman appear to be directly due to a pathologically high plasma testosterone level, and since the excretion of urinary 17-ketosteroids does not correlate with the plasma level or the production rate of testosterone, combined adrenal and ovarian function tests based on urinary 17-ketosteroid determinations such as have been proposed by SEGRE (1967) appear to be of doubtful value. Nevertheless, determination of the 17-ketosteroids is useful for establishing the organ of origin of elevated androgens in the woman. Whenever a pathologically elevated plasma testosterone is of adrenal origin, elevated levels of urinary 17-ketosteroids are found. When these are not clearly suppressible by dexamethasone, 2 mg daily, an adrenal cortical tumor must be suspected. On the other hand, even considerably increased testosterone production by the ovaries does not lead to elevated levels of 17-ketosteroids. However, an exception is found in the Stein-Leventhal syndrome, in which a relatively high proportion of the urinary 17-ketosteroids is of ovarian origin and can be stimulated by chorionic gonadotropin when the adrenal cortex is blocked by dexamethasone (MAHESH, 1964).

8. Indirect Evaluation of Adrenocortical Function

Direct assessment of adrenocortical function is now possible by determination of urinary or plasma corticosteroids in stimulation and suppression tests, so that the indirect function tests have lost a great deal of their importance.

An exact determination of aldosterone is so time-consuming, however, that the aldosterone production rate is often indirectly assessed by sodium and potassium balance studies or by determination of salivary or sweat electrolytes as screening procedures.

An estimation of mineralocorticoid production is also possible by the determination of fecal electrolytes. The unpleasant analysis of stool aliquots can be avoided by a simple *in-vivo* dialysis procedure (RICHARDS, 1969). Mineralocorticoid production can also be evaluated by the determination of the difference in electric potential between the rectal mucosa and the perianal skin (EDMONDS, 1970).

Sodium restriction, potassium loading, and insulin tolerance tests are dangerous and unnecessary screening procedures for adrenocortical insufficiency. The insulin tolerance test may lead to normal results in untreated Addisonian patients in whom some glycogen stores are still available and the blood sugar may spontaneously return to normal values.

Instead of the straining Robinson-Power-Kepler tests, we prefer to assess the single parameters, i.e. the capacity of diuresis and clearances, to a combined index. A simple water tolerance test is occasionally justified with due consideration for the risk of hypotonic hydration and with appropriate precautions.

a) Water Tolerance Test According to VOLHARD, *as Modified by* SOFFER *(1952)*

Method: No fluid or food is allowed after the evening meal the day before the test.

At 7 a.m. on the day of the test, urine is voided and discarded. The patient then drinks 1.5 liters of water or of tea with lemon and saccharine within 15 to 45 min. Urine is collected from 7 a.m. to 12 noon and the volume is measured. The patient is lying down or sitting when not voiding urine.

Interpretation. Normally, at least 800 ml are excreted in five hours. In adrenal or pituitary insufficiency, the urine volume excreted is less than 800 ml. A pathologic result of the test can be due to dehydration, tendency to edema or to cardiac or liver diseases or hypothyroidism.

b) Salivary Electrolytes

The sodium and potassium concentrations in the saliva are controlled among other factors by the adrenal cortex. Valid conclusions about mineralocorticoid activity can be drawn from these values when the other influences (time of day, duration of stimulation, secretion rate, salt content of the food) are taken into account. The test allows indirect estimation of the aldosterone production rate.

Method and Calculation (PRADER, 1955). An essential condition is a normal salt intake (10 g of NaCl per day) on the preceding days. A piece of unbreakable paraffin, the size of a hazel nut, is chewed for 10 min between 6 and 8 a.m. in the fasting state and before brushing the teeth. The patient is told not to swallow and to collect all the saliva in a glass container. The saliva of the first and second five-minute periods is collected separately, and only the saliva from the second period is examined. The volume (ml per 5 min) and the sodium and potassium concentrations are determined. The

sodium and potassium concentration and the sodium-potassium ratio are then corrected to a standard secretion rate (10 ml per 5 min = 2 ml per min) by the following formulas:

Na_k = Na + 1.6 (10–vol/5 min),
K_k = K,
$(Na/K)_k$ = Na/K + 0.1 (10–vol/5 min).

The normal values for adults and children are (mean ± 2 standard deviations):

Na_k = 20–36 mEq/l,
K = 11–27 mEq/l,
$(Na/K)_k$ = 0.6–2.1.

In pathologic conditions, the sodium values are changed more than the potassium values and the two ions usually move in opposite directions. Increased sodium and sodium/potassium values are indicative of adrenocortical failure, decreased values indicate mineralocorticoid excess. The test is not reliable when the serum electrolytes are conspicuously altered. A sodium/potassium ratio of less than 0.25 strongly indicates a primary or secondary aldosteronism. An excessive aldosterone production is unlikely when the value is above 0.5.

c) Evaluation of Sodium and Potassium Excretion in the Sweat

Like the sodium and potassium excretion in saliva, the secretion rate of sodium and potassium in sweat provides valid information on the production of mineralocorticoid hormones. With due consideration of the sweat secretion rate, the determination of sweat electrolytes can be used as a screening procedure for primary or secondary aldosteronism and is of value for mass screening of hypertensives. A reliable procedure has been developed by GRANDCHAMP (1968, 1969) and has been evaluated as a diagnostic tool for the various types of hypermineralocorticoidism.

Principle. After 5 min of pilocarpin iontophoresis on the forearm (GIBSON, 1959), sweat is collected for 20 min under a flat cover and drawn up into glass capillaries. The sweat secretion rate is determined by weighing the capillaries, and sodium and potassium concentrations are measured by flame spectrophotometry.

Results:

x = sweat secretion rate in µg/20 min/13.8 cm^2.
y = sodium/potassium ratio.

Normals:

$y = 0.03x + 1.81$
(± 2 standard deviations =) ± 2.60.

Experimental secondary aldosteronism:

$y = 0.02x + 0.5 \pm 1.3$.

Experimental primary aldosteronism:

$y = 0.004x + 1.37 \pm 0.62$.

In two cases of primary aldosteronism and in one case of renovascular hypertension, the sodium/potassium values were clearly in the range of results observed in experimental aldosteronism.

d) Determination of Total Body Potassium and Sodium

α) Determination of Potassium^{40} by Whole Body Counting

Natural potassium contains up to 0.0119% the radioactive isotope ^{40}K, a β- and γ-emitter with a half-life of 1.4×10^9 years. Determination of this isotope by whole-body counting is a relatively reliable index of total body potassium. Counting with the apparatus of JOYET (1968), which allows an optimum in couting geometry, takes 45 min. The instrumental measuring error is ±3.5–5%. Values determined in the same subject after intervals of several weeks vary by ±7%. Individual sex- and age-dependent variations appear to be mainly due to the variable portion of adipose tissue in the whole body mass. Adipose tissue has a very low potassium content.

Normal values according to JOYET (1968):

Men:
Age 20 years: 2.14 ± 0.02 g of K/kg
25–34 years: 1.89 ± 0.07 g of K/kg;
35–44 years: 1.94 ± 0.10 g of K/kg;
Above 45 years: 1.82 ± 0.06 g of K/kg;
Women:
Age 20 years: 1.58 ± 0.02 g of K/kg:

In patients with primary aldosteronism, the whole-body potassium was decreased by 20–50%.

β) Determination of Exchangeable Potassium and Sodium

For this determination two artificial short-life isotopes, ^{42}K and ^{24}Na, are used. By the determination of the potassium which is exchangeable with ^{42}K, 90 to 95% of the whole-body potassium is measured, whereas the sodium exchangeable with ^{24}Na is about 80

to 85% of the total body sodium. The specific activity of potassium or sodium in body fluids reaches a maximum 18 to 24 hours after the injection of a tracer dose of ^{42}KCl or $^{24}NaCl$ and remains constant for approximately 24 hours.

Exchangeable Potassium. According to the procedure of CORSA (1950), approximately 100 μCi of ^{42}KCl are given by i.v. injection. Urine is collected for 24 hours for the determination of ^{42}K-excretion. After 23, 24 and 25 hours, potassium (^{39}K) is measured in a fresh urine portion by flame photometry and the ^{42}K-activity is counted; the specific activity of urinary potassium is calculated from these two values. The exchangeable potassium can be calculated from this specific activity and from the radioactivity remaining in the body by the following formula:

$$\text{exchangeable potassium} = (^{42}K \text{ injected} - ^{42}K \text{ excreted}) \times \frac{^{39}K}{^{42}K}.$$

Normal values according to DEMANET (1958):

Men: 53 ± 5 mEq/kg;
Women: 42 ± 7 mEq/kg.

Exchangeable Sodium. According to FORBES (1951), the determination is performed by practically the same procedure as the one used for the determination of exchangeable potassium. About 100 μc of $^{24}NaCl$ are injected and the excretion in the 24-hour urine is determined. After 24 hours, the sodium concentration (^{23}Na) is determined by flame photometry and ^{24}Na is counted not in the urine, but in the serum.

Normal values according to DEMANET (1958):

Men: 43 ± 5 mEq/kg;
Women: 39 ± 5 mEq/kg.

References

Reviews

BAULIEU, E. E., ROBEL, P. (eds.): Aldosterone. Oxford: Blackwell 1964.

DOHM, G.: Die Nebenniere im Kindesalter. Berlin-Göttingen-Heidelberg: Springer 1965.

EIK-NES, K. B., HALL, P. F.: Secretion of steroid hormones in vivo. Vitam. and Horm. **23**, 153 (1965).

EISENSTEIN, A. B.: The adrenal cortex. Boston: Little, Brown & Co. 1967.

GRAY, C. H., BACHARACH, A. L. (eds.): Hormones in blood, vols. 1 and 2, 2nd ed. New York: Academic Press 1967.

HÜBENER, H. J., STAIB, W. H.: Biochemie der Nebennierenrinden-Hormone. Stuttgart: Georg Thieme 1965.

JAMES, V. H. T., LANDON, J.: The investigation of hypothalamic-pituitary-adrenal function. Cambridge: University Press 1968.

MCKERNS, K. W.: Functions of the adrenal cortex. Amsterdam: North Holland Publ. Comp. 1968.

MOON, H. D.: The adrenal cortex. New York: Hoeber 1961.

PRUNTY, F. T. G.: Chemistry and treatment of adrenocortical diseases. Springfield: Thomas 1964.

SOFFER, L. J., DORFMAN, R. I., GABRILOVE, J. L.: The human adrenal gland. Philadelphia: Lea & Febiger 1961.

SYMINGTON, TH.: Functional pathology of the human adrenal gland. Edinburgh-London: Livingstone 1969.

THORN, G. W., JENKINS, D., LAIDLAW, J. C., GOETZ, F. C., DINGMAN, J. F., ARONS, W. L., STREETEN, D. H. P., MCCRACKEN, B. H.: Pharmacological aspects of adrenocortical steroids and ACTH in man. New Engl. J. Med. **248**, 232 (1953). With review of literature to 1953.

YATES, F. E., URQUHART, J.: Control of plasma concentration of adrenocortical hormones. Physiol. Rev. **42**, 359 (1962).

ZIMMERMAN, W.: Chemie und Stoffwechsel der Steroidhormone. In: Handbuch der inneren Medizin, Bd. VII/I. Berlin-Göttingen-Heidelberg: Springer 1955.

Embryology and Normal Anatomy

BARGMANN, W.: Histologie und mikroskopische Anatomie, 2. Aufl. Stuttgart: Georg Thieme 1956.

EDMUNDS, H. W.: Pituitary, adrenal and thyroid in cyclopia. Arch. Path. **50**, 727 (1950).

FLINT, J. M.: The blood-vessels, angiogenesis, organogenesis, reticulum and histology of the adrenal. Johns Hopk. Hosp. Rep. **9**, 153 (1900).

FORBES, TH. R.: Studies on the reproductive system of the alligator. IV. Observations of the development of the gonad, the adrenal cortex and the Mülleran duct. Contr. Embryol. Carneg. Instn **28**, 157 (1940).

JOST, A.: The age factor in the castration of the male rabbit fetuses. Proc. Soc. exp. Biol. (N.Y.) **66**, 302 (1947).

— Influence de la décapitation sur le développement du tractus génital et des surrénales de l'embryon dc lapin. C. R. Soc. Biol (Paris) **142**, 273 (1948).

— La physiologie de l'hypophyse foetale. Biol. méd. (Paris) **39**, 577 (1951).

KITCHELL, R. L.: Reciprocal relation between the hypophysis and adrenals in fetal rats: effects of unilateral adrenalectomy and of implanted cortisone, doca and sex hormones. Endocrinology **50**, 83 (1952).

—, WELLS, L. J.: Functioning of the hypophysis and adrenals in fetal rats: effects of the hypophysectomy, adrenalectomy, castration, injected ACTH and implanted sex hormones. Anat. Rec. **112**, 561 (1952).

MOORE, C. R.: Embryonic sex hormones and sexual differentiation. Springfield, Illinois: Ch. C. Thomas 1948.

PROPST, A., MÜLLER, O.: Die Zonen der Nebennierenrinde der Ratte. Z. Zellforsch. **75**, 404 (1966).

RAYNAUD, A., FRILLEY, M.: Développement intra-uterin des embryons de souris dont les ébauches de l'hypophyse ont été détruites au moyen des rayons X, au 13e jour de la gestation. II. Développement des capsules surrénales. C. R. Acad. Sci. (Paris) **230**, 331 (1950).

ROTTER, W.: Die Entwicklung der fetalen und kindlichen Nebennierenrinde. Virchows Arch. path. Anat. **316**, 590 (1949).

SMITH, P. E.: The pigmentary, growth and endocrine disturbances in the anuran tadpole by the early ablation of the pars buccalis of the hypophysis. Amer. Anat., Mem. No. 11, **1920**.

— Increased skeletal effects in A. P. growth-hormone injections by administration of thyroid in hypophysectomized, thyro-parathyroidectomized rats. Proc. Soc. exp. Biol. (N. Y.) **30**, 1252 (1933).

— SMITH, I. B.: The function of the lobes of the hypophysis as indicated by replacement therapy with different portions of the gland. Endocrinology **7**, 579 (1923).

STARCK, D.: Embryologie. Stuttgart: Georg Thieme 1955.

TOBIN, C. E.: The influence of adrenal destruction on the prenatal development of the albino. Amer. J. Anat. **65**, 151 (1939).

TONUTTI, E.: Experimentelle Untersuchungen zur Pathophysiologie der Nebennierenrinde. Verh. dtsch. Ges. Path. **36**, 123 (1953).
WELLS, L.J.: Effects of injections of equine gonadotrophin upon gonads and adrenals of fetal rats. Proc. Soc. exp. Biol. (N. Y.) **62**, 250 (1946).
— Some experimental evidence of production of adrenotrophin by the fetal hypophysis. Proc. Soc. exp. Biol. (N.Y.) **68**, 487 (1948).
— FRALICK, R.L.: Production of androgen by the testes of fetal rats. Amer. J. Anat. **89**, 63 (1951).
YAKAITIS, A.A., WELLS, L.J.: Hypophysis-adrenal system in the fetal rat: Adrenals in fetuses subjected to cortisone, hydrocortisone, DCA, hypophyseoprivia and growth hormone. Amer J. Anat. **98**, 205 (1956).
YATES, R.D.: Fine structural observation on untreated and ACTH treated adrenocortical cells of the zona reticularis of syrian hamsters. Z. Zellforsch. **66**, 384 (1965).

Biochemistry

Steroidhormones, Stereoisomerism and Nomenclature

FIESER, L.F., FIESER, M.: Steroids. New York: Reinhold 1959.
HIRSCHMANN, H.: Chemistry of steroid hormones. In: Pincus and Thimann, The hormones, vol. III. New York: Academic Press 1955.
IUPAC-IUB 1967 Revised tentative Rules for steroid Nomenclature. Biochim. biophys. Acta **164**, 453 (1968).
KLYNE, W.: The chemistry of the steroids. London: Methuen & Co Ltd., 1957.
SHOPEE, C.W.: Steroid configuration. Vitam. and Horm. **8**, 255 (1950).
ZIMMERMANN, W.: Chemie und Stoffwechsel der Steroidhormone. In: Handbuch der inneren Medizin, Bd. VII/I. Berlin-Göttingen-Heidelberg: Springer 1955.

The Adrenocortical Hormones

Cortexolone

COPE, C.L., DENNIS, P.M., PEARSON, J.: Some factors determining the adrenal response to metyrapone (SU 4885). Clin. Sci. **30**, 249 (1966).
HERTOGH, R. DE, HOET, J.J., MATERAZZI, F., EKKA, E.: The fate of 1,2-^{3}H Cortexolone in man. Acta endocr. (Kbh.) **47**, 165 (1964).

Deoxycorticosterone

HARRIS, J.J., HOEGEL, C., CRANE, M.G.: Deoxycorticosterone secretion rates before and during metyrapone administration in normal subjects. J. clin. Endocr. **27**, 106 (1967).

18-Hydroxycorticosterone

ULICK, S., NICOLIS, G.L., VETTER, K.K.: Relationship of 18-hydroxycorticosterone to aldosterone. In: E.E. BAULIEU and P. ROBEL (ed.), Aldosterone. Oxford: Blackwell **3**, 1964.

Dehydroepiandrosterone-Sulfate

BAULIEU, E.-E., CORPÉCHOT, C., DRAY, F., EMILIOZZI, R., LEBEAU, M.-C., MAUVAIS-JARVIS, P., ROBEL, P.: An adrenal-secreted "androgen": dehydroisoandrosteron sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates in man. Recent Progr. Hormone Res. **21**, 411 (1965).

Estrogens

BROWN, J.C., FALCONER, C.W.A., STRONG, J.A.: Urinary oestrogens of adrenal origin in women with breast cancer. J. Endocr. **19**, 52 (1959).
TAMM, J., APOSTOLAKIS, M., VOIGT, K.D.: The effects of ACTH and HCG on the urinary excretion of male patients with various endocrine disorders. Acta endocr. (Kbh.) **53**, 61 (1966).

Biosynthesis of Adrenocortical Hormones

BLOCH, K.: Biosynthesis of cholesterol. In: G. PINCUS, Hormones and atherosclerosis. New York: Academic Press 1959.
— Die Biosynthese des Cholesterins (Nobel-Vortrag). Angew. Chem. **77**, 944 (1965).
HAYANO, M., SABA, N., DORFMAN, R.I., HECHTER, O.: Some aspects of the biogenesis of adrenal steroid hormones. Recent Progr. Hormone Res. **12**, 79 (1956).
HEARD, R.D.H., BILG, E.G., CANN, M.C., HELLINEK, P.H., O'DONNEL, V.J., RAO, R.G., WEBB, J.L.: Biogenesis of the sterols and steroid hormones. Recent Progr. Hormone Res. **12**, 45 (1956).
HECHTER, O., PINCUS, G.: Genesis of the adreno-cortical secretion. Physiol. Rev. **34**, 459 (1954).
KAHNT, F.W., NEHER, R.: Über die adrenale Steroid-Biosynthese *in vitro*. I. Umwandlung endogener und exogener Vorstufen im Nebennieren-Homogenat des Rindes. Helv. chim. Acta **48**, 1457 (1965).
KRUM, A.A., MORRIS, M.D., BENNET, L.L.: Role of cholesterol in the *in vivo* biosynthesis of adrenal steroids by the dog. Endocrinology **74**, 543 (1964).
NEHER, R., WETTSTEIN, A.: Occurence of Δ^5-3β-Hydroxyosteroids in adrenal and testicular tissue. Acta endocr. (Kbh.) **35**, 1 (1960).
PINCUS, G.: The biosynthesis of adrenal steroids. Ann. N.Y. Acad. Sci. **61**, 283 (1955).
— ROMANOFF, E.B.: The synthesis of corticosteroids by the human adrenal cortex. Ciba Found. Coll. Endocr. **8**, 97 (1955).
POPJAK, G.: Some aspects of the biosynthesis of cholesterol from mevalonic acid. In: G. PINCUS, Hormones and atherosclerosis. New York: Academic Press 1959.
SAMUELS, L.T., UCHIKAWA: Biosynthesis of adrenal steroids In: A.B. EISENSTEIN (ed.), The adrenal cortex. Boston: Little, Brown & Co. 61, 1967.
STAUDINGER, H.J.: Biosynthese der Steroidhormone. In: Hormone und ihre Wirkungsweise. Berlin-Göttingen-Heidelberg: Springer 1955.
WERBIN, H., CHAIKOFF, I.L.: Utilization of adrenal gland cholesterol for synthesis of cortisol by the intact normal and ACTH-treated guinea pig. Arch. Biochem. **93**, 476 (1961).
WETTSTEIN, A.: Aldosteron. In: LETTRÉ-INHOFFEN-TSCHESCHE, Über Sterine, Gallensäuren und verwandte Stoffe, Bd. 2. Stuttgart 1956.
— Biosynthèse des hormones stéroides. Experientia (Basel) **17**, 329 (1961).
— Chemie des Aldosterons. Verh. dtsch. Ges. inn. Med. **68**, 582 (1962).

Transport of Adrenocortical Hormones

BERNSTEIN, S., SOLOMON, S.: Chemical and biological aspects of steroid conjugation. Berlin-Heidelberg-New York: Springer 1970.

BUSH, I. E.: The physicochemical state of cortisol in blood. Ciba Found. Coll. Endocr. **11**, 263 (1957).
DAUGHADAY, W. H.: Steroid protein interactions. Phys. Rev. **39**, 885 (1959).
— The binding of corticosteroids by plasma protein. In: A. B. EISENSTEIN (ed.), The adrenal cortex. Boston: Little, Brown & Co 1967.
— MARIZ, I. K.: The binding of steroid hormones by plasma proteins. In: Biological activities of steroids in relation to cancer (eds. G. PINCUS und E. P. VOLLMER). New York: Academic Press 1960.
MEYER, CL. J., LAYNE, D. S., TAIT, J. F., PINCUS, G.: The binding of aldosterone to plasma proteins in normal, pregnant and steroid-treated women. J. clin. Invest. **40**, 1663 (1961).
MIGEON, C. J., LAWRENCE, B., BERTRAND, J., HOLLMAN, G. H.: In vivo distribution of some 17-hydroxycosteroids between the plasma and red blood cells of man. J. clin. Endocr. **19**, 1411 (1959).
MILLS, J. H., SCHEDEL, H. P., CHEN, P. S., BARTTER, F. C.: The effect of estrogen administration on the metabolism and protein binding of hydrocortison. J. clin. Endocr. **20**, 515 (1960).
PLAGER, J. E., SCHMIDT, K. G., STAUBITZ, W. J.: Increased unbound cortisol in the plasma of estrogen-treated subjects. J. clin. Invest. **43**, 1066 (1964).
SANDBERG, A. A., SLAUNWHITE, W. R., JR.: Transcortin: A corticosteroid-binding protein of plasma. V. *In vitro* inhibition of cortisol metabolism. J. clin. Invest. **42**, 51 (1963).
— — ANTONIADES, H. N.: The binding of steroids and steroid conjugates to human plasma protein. Recent Progr. Hormone Res. **13**, 209 (1957).
— — CARTER, A. C.: Transcortin: A corticosteroid-binding protein of plasma. III. The effect of various steroids. J. clin. Invest. **39**, 1914 (1960).
SLAUNWHITE, W. R., JR., LOCKIE, G. N., BACK, N., SANDBERG, A. A.: Transcortin: a corticosteroid binding protein. IV. Inactivity *in vivo* of transcortin-bound cortisol Science **135**, 1062 (1962).

Inactivation, Catabolism and Excretion of Adrenocortical Hormones

BEISEL, W. R., COS, J. J., HORTON, R., CHAO, P. Y., FORSHAM, P. H.: Physiology of urinary cortisol excretion. J. clin. Endocr. **24**, 887 (1964).
BERLINER, D. L., DOUGHERTY, TH. F.: Hepatic and extrahepatic regulation of corticosteroids. Pharmacol. Rev. **13**, 329 (1961).
DORFMAN, R. I.: Neutral steroid hormon metabolites. Recent Progr. Hormone Res. **9**, 5 (1954).
— Special aspects of adrenocortical steroid metabolism and generalizations. Ciba Found. Coll. Endocr. **8**, 112 (1955).
FRANTZ, A. G., KATZ, F. H., JAILER, J. W.: 6β-Hydroxycortisol and other polar corticosteroids: measurement and significance in human urine. J. clin. Endocr. **21**, 1290 (1961).
ISSELBACHER, K. J.: Enzymatic mechanisms of hormone metabolism. II. Mechanism of hormonal glucuronide formation. Recent Progr. Hormone Res. **12**, 134 (1956).
LIPMAN, M. M., KATZ, F. H., JAILER, J. W.: An alternate pathway for cortisol metabolism: 6-β-hydroxycortisol production by human tissue slices. J. clin. Endocr. **22**, 268 (1962).
MIGEON, C. J., SANDBERG, A. A., DECKER, H. A., SMITH, D. F., PAUL, A. C., SAMUELS, T.: Metabolism of 4-C^{14}-corticosterone in man: body distribution and rates of conjugation. J. clin. Endocr. **16**, 1137 (1956).
— — PAUL, A. C., SAMUELS, T.: Metabolism of 4-C^{14}-corticosterone in man. J. clin. Endocr. **16**, 1291 (1956).
PETERSON, R. E.: Plasmacorticosterone and hydrocortisone levels in man. J. clin. Endocr. **17**, 1150 (1957).
— The influence of the thyroid on adrenal cortical function. J. clin. Invest. **37**, 736 (1958).
— Miscible pool and turnover rate of adrenal corticosteroids in man. Recent Progr. Hormone Res. **15**, 231 (1959).
— Adrenocortical steroid metabolism and adrenal cortical function in liver disease. J. clin. Invest. **39**, 320 (1960).
— PIERCE, C. E.: The metabolism of corticosterone in man. J. clin. Invest. **39**, 741 (1960).
— — WYNGAARDEN, J. B., BRODIE, B. B.: The physiological disposition and metabolic fate of cortisone in man. J. clin. Invest. **36**, 1301 (1957).
— WYNGAARDEN, J. B.: The miscible pool and turnover rate of hydrocortisone in man. J. clin. Invest. **35**, 552 (1956).
SAMUELS, L. T.: Metabolism of steroid hormones. In: D. M. GREENBERG, Metabolic pathways, vol. 1, New York: Academic Press 1960.
SANDBERG, A. A., EIK-NES, K., MIGEON, C. J., SAMUELS, L. T.: Metabolism of adrenal steroids in dying patients. J. clin. Endocr. **16**, 1001 (1956).
SAYERS, G. E., GLENN, M., SYDNOR, K. L., LIPSCOMB, M., SWEAT, M. L., KELLY, L. W., LEVY, R. P., MCK. JEFFERIES, W.: Plasma and urinary steroids after hydrocortisone infusion. J. clin. Invest. **34**, 1600 (1955).
TAIT, J. F., TAIT, S. A. S., LITTLE, B., LAUMAS, K. R.: The disapearence of 7-H^3-d-Aldosterone in the plasma of normal subjects. J. clin. Invest. **40**, 72 (1961).
TOMKINS, G. M.: Enzymatic mechanism of hormone metabolism. I. Oxidation-reduction of the steroid nucleus. Recent Progr. Hormone Res. **12**, 125 (1956).
UNDERWOOD, R. H., TAIT, J. F.: Purification, partial characterization and metabolism of an acid labile conjugate of aldosterone. J. clin. Endocr. **24**, 1110 (1964).

Chemistry of Corticotropin (ACTH) and the Melanotropins (MSH)

BRADLOW, H. L., FUKUSHIMA, D. K., GALLAGHER, T. F., HELLMAN, L., HOFMANN, K., LI, CH. H., ROSENFELD, R. S., SPENCER, H., ZUMOFF, B.: Adrenal stimulation by adrenocorticotropic peptides. J. clin. Endocr. **23**, 792 (1963).
HOFMANN, K.: Chemistry and function of polypeptide hormones. Ann. Rev. Biochem. **31**, 213 (1962).
— YAJIMA, H.: Synthetic pituitary hormones. Recent Progr. Hormone Res. **18**, 31 (1962).
LI, CH. H.: Synthesis and biological properties of ACTH peptides. Recent Progr. Hormone Res. **18**, 1 (1962).
— The ACTH-molecule. Sci. Amer. **209**, 47 (1963).
— OELOFSEN, W.: The chemistry and biology of ACTH and related peptides. In: A. B. EISENSTEIN (ed.), The adrenal cortex, p. 185. Boston 1967.
NEY, R. L., SHIMIZU, N., NICHOLSON, W. E., ISLAND, D. P., LIDDLE, G. W.: Correlation of plasma ACTH concentration with adrenocortical response in normal human subjects, surgical patients and patients with Cushing's disease. J. clin. Invest. **42**, 1669 (1963).
SCHWYZER, R.: Structure-activity relationship among physiologically active polypeptides. Proc. 1st internat. pharmacol. meeting, vol. 7. Oxford: Pergamon Press 1963.
— Synthetische Polypeptide mit physiologischer Wirkung. Ergebnisse der Physiologie, Bd. 53. Berlin-Göttingen-Heidelberg: Springer 1963.
— COSTOPANAGIOTIS, A., SIEBER, P.: Zwei Synthesen des α-Melanotropins (α-MSH) mit Hilfe leicht entfernbarer Schutzgruppen. Helv. chim. Acta **46**, 871 (1963).
— KAPPELER, H.: Synthese eines Tetracosapeptides mit hoher corticotroper Wirksamkeit: β^{1-24}-Corticotropin. Helv. chim. Acta **46**, 1550 (1963).
— SIEBER, P.: Die Totalsynthese des β-Corticotropins (Adrenocorticotropes Hormon; ACTH). Helv. chim. Acta **49**, 134 (1966).

WALSER, A., MÜLLER, TH.: Adrenocorticotropic effect in humans of a synthetic β^{1-24}-corticotrophine derivative. Experientia (Basel) **24**, 74 (1968).
YALOW, R.S., BERSON, S.A.: Characteristics of "big ACTH" in human plasma and pituitary extracts. J. clin. Endocr. **36**, 415 (1973).

Physiology

Reviews

BUSH, I.E.: Chemical and biological factors in the activity of adrenocortical steroids. Pharmacol. Rev. **14**, 317 (1962).
DEANE, H.W.: The anatomy, chemistry and physiology of adrenocortical tissue. In: Handbuch der experimentellen Pharmakologie, Bd. 14,1. The adrenocortical hormones. Berlin-Göttingen-Heidelberg: Springer 1962.
THORN, G.W., u.a. (Literatur bis 1953): Pharmacological aspects of adrenocortical steroids and ACTH in man. New Engl. J. Med. **248**, 232 (1953).
YATES, F.E., URQUHART, J.: Control of plasma concentrations of adrenocortical hormones. Physiol. Rev. **42**, 335 (1962).

Effects of Corticotropin and MSH

FAIN, J.N.: Adrenergic blockade of hormone induced lipolysis in isolated fat cells. Ann. N.Y. Acad. Sci. **139**, 879 (1967).
— KOVACEV, V.P., SCOW, R.O.: Effect of growth hormone and dexamethasone on lipolysis and metabolism in isolated fat cells of the rat. J. biol. Chem. **240**, 3522 (1965).
FERGUSON, J.J.: Protein synthesis and adrenocorticotropin responsiveness. J. biol. Chem. **238**, 2754 (1963).
FIALA, S., SPROUL, E., FIALA, A.: Action of corticotropin (ACTH) on nucleic acids and subcellular elements of adrenal cortex. J. biophys. biochem. Cytol. **2**, 115 (1956).
GARREN, L.D., GILL, G.N., MASUI, H., WALTON, G.M.: On the mechanism of action of ACTH. Recent Progr. Hormone Res. **27**, 433 (1971).
— NEY, R.L., DAVIS, W.W.: Studies on the role of protein synthesis in regulation of corticosterone production by adrenocorticotropic hormone *in vivo*. Proc. nat. Acad. Sci.(Wash.) **53**, 1443 (1965).
GRANT, J.K., SYMINGTON, T., DUGUID, W.P.: Effect of adrenocorticotropic therapy on the *in vitro* 11β-hydroxylation of deoxycorticosterone by human adrenal homogenates. J. clin. Endocr. **17**, 933 (1957).
HAYNES, R.C., JR., BERTHET, L.: Studies on mechanism of action of adrenocorticotropic hormone. J. biol. Chem. **225**, 115 (1957).
— SUTHERLAND, E.W., RALL, T.W.: The role of cyclic adenylic acid in hormone action. Recent Progr. Hormone Res. **16**, 121 (1960).
HILF, R.: The mechanism of action of ACTH. New Engl. J. Med. **273**, 798 (1965).
JENNY, M., MÜLLER, A., MACH, R.S.: Effects chimiques et métaboliques d'un nouveau polypeptide à action adrénocorticotrope (tétracosapeptide). Schweiz. med. Wschr. **93**, 766 (1963).
KARABOYAS, G.C., KORITZ, S.B.: Identity of the site of action of 3',5'-adenosine monophosphate and adrenocorticotropic hormone in corticoidogenesis in rat adrenal and beef adrenal cortex sclices. Biochemistry **4**, 462 (1965).
NELSON, D.H., HUME, D.M.: Corticosteroid secretion in the adrenal venous blood of the hypophysectomized dog as an assay for ACTH. Endocrinology **57**, 184 (1955).
NUGENT, CH.A., EIK-NES, K., SAMUELS, L.T., TYLER, F.H.: Changes in plasma levels of 17-Hydroxycorticosteroids during the intravenous administration of adrenocorticotropin. J. clin. Endocr. **19**, 334 (1959).
— MACDIARMID, W.D., NELSON, A.R., TYLER, F.H.: Rate of adrenal cortisol production in response to maximal stimulation with ACTH. J. clin. Endocr. **23**, 684 (1963).
RENOLD, A.E., JENKINS, D., FORSHAM, P.H., THORN, G.W.: The use of intravenous ACTH: a study in quantitative adrenocortical stimulation. J. clin. Endocr. **12**, 763 (1952).
SCHULER, W., SCHÄR, B., DESAULLES, P.: Pharmakologie des Synacthen. Schweiz. med. Wschr. **93**, 1027 (1963).
SCRIBA, P.C.: Zum biochemischen Wirkungsmechanismus des adreno-corticotropen Hormons: Einfluß auf die Synthese von Eiweiß in der Nebenniere. Klin. Wschr. **42**, 463 (1964).
STONE, D., HECHTER, O.: Studies of ACTH action in perfused bovine adrenals: the site of action of ACTH in corticosteroidogenesis. Arch. Biochem. **51**, 457 (1954).
VAUGHAN, M., STEINBERG, D.: Glyceride biosynthesis, glyceride breakdown and glycogen breakdown in adipose tissue: Mechanism and regulation. In: Handbook of physiology. Sect. 5: Adipose tissue, A.E. RENOLD and G.F. CAHILL, JR. (eds.), p. 239. Washington, D.C.: Amer. physiol. Soc. 1965.
WHITE, J.E., ENGEL, F.L.: Lipolytic action of Corticotropin on rat adipose tissue *in vitro*. J. clin. Invest. **37**, 1556 (1958).

Effects of ACTH and MSH on the Pigment Formation

ABE, K., NICHOLSON, W.E., LIDDLE, G.W., ORTH, D.N., ISLAND, D.P.: Normal and abnormal regulation of β-MSH in man. J. clin. Invest. **48**, 1580 (1969).
FLÜCKIGER, E.: Zur Biologie der Farbwechselhormone. Verh. Naturf. Ges. Basel **73**, 194 (1962).
HARRIS, I.: A melanocyte stimulating hormone from the human pituitary gland. Ciba Found. Coll. Endocr. **13**, 266 (1963).
LEE, T.H., LERNER, A.B., BUETTNER-JANUSCH, V.: Adrenocorticotropic hormone and melanocyte-stimulating hormone from human pituitary glands. Ciba Found. Coll. Endocr. **13**, 251 (1960).
LERNER, A.B.: Hormones and skin color. Sci. Amer. **205**, 199 (1961).
— MCGUIRE, J.S.: Melanocyte-stimulating hormone and adrenocorticotropic hormone. Their relation to pigmentation. New Engl. J. Med. **270**, 539 (1964).
SULMAN, F.G., ASTRAIR, Z., ECKSTEIN, B., KHAZAN, N., PFEIFFER, Y.: Separation of ACTH and MSH fractions from pituitaries of different species. Proc. Soc. exp. Biol. (N.Y.) **112**, 202 (1963).

Control of Aldosterone Secretion

BLAIR-WEST, J.R., COGHLAN, J.P., DENTON, D.A., GODING, J.R., MUNRO, J.A., PETERSON, R.E., WINTOUR, M.: Humoral stimulation of adrenal cortical secretion. J. clin. Invest. **41**, 1606 (1962).
— — — — WINTOUR, M., WRIGHT, R.D.: The control of aldosterone secretion. Recent Progr. Hormone Res. **19**, 311 (1963).
— — — MUNRO, J.A., WINTOUR, M., WRIGHT, R.D.: The effect of bilateral nephrectomy and midcolicular decerebration with pinealectomy and hypophysectomy on the corticosteroid secretion of sodium-deficient sheep. J. clin. Invest. **43**, 1576 (1964).

DAVIS, J. O.: A critical evaluation of the role of receptors in the control of aldosterone secretion and sodium excretion. Progr. cardiovasc. Dis. **4**, 27 (1961).
— Mechanisms regulating the secretion and metabolism of aldosterone in experimental hyperaldosteronism. Recent Progr. Hormone Res. **17**, 293 (1961).
— Aldosterone and angiotensin. J. Amer. med. Ass. **188**, 1062 (1964).
— The regulation of aldosterone secretion. In: The adrenal cortex (A. B. EISENSTEIN, ed.), p. 203. Boston: Little, Brown & Co. 1967.
— CARPENTER, CH. C. J., AYERS, C. R., HOLMAN, J. E., BAHN, R. C.: Evidence for secretion of an aldosterone-stimulating hormone by the kidney. J. clin. Invest. **40**, 684 (1961).
— URQUHART, J., HIGGINS, J. T., JR.: The effects of alterations of plasma sodium and potassium concentration on aldosterone secretion. J. clin. Invest. **42**, 597 (1963).
DAVIS, W. W., BURWELL, L. R., CASPER, A. G. T., BARTTER, F. C.: Sites of action of sodium depletion on aldosterone biosynthesis in the dog. J. clin. Invest. **47**, 1425 (1968).
GANONG, W. F., BIGLIERI, E. G., MULROW, P. J.: Mechanisms regulating adrenocortical secretion of aldosterone and glucocorticoids. Recent Progr. Hormone Res. **22**, 381 (1966).
GENEST, J.: Angiotensin, aldosterone and human arterial hypertension. Canad. med. Ass. J. **84**, 403 (1961).
GORDON, R., KÜCHEL, O., LIDDLE, G. W., ISLAND, D. P.: Role of the sympathetic nervous system in regulating renin and aldosterone production in man. J. clin. Invest. **46**, 599 (1967).
GROSS, F.: Renin und Hypertensin, physiologische oder pathologische Wirkstoffe? Klin. Wschr. **36**, 693 (1958).
— Die Steuerung der Aldosteronsekretion. Schweiz. med. Wschr. **89**, 1 (1959).
KAPLAN, N. M., BARTTER, F. C.: The effect of ACTH, renin, angiotensin II and various precursors on biosynthesis of aldosterone by adrenal slices. J. clin. Invest. **41**, 715 (1962).
LARAGH, J. H., ANGERS, M., KELLY, W. G., LIEBERMAN, S.: Hypotensive agents and pressor substances. The effect of epinephrine, norepinephrine, angiotensin II, and others on the secretory rate of aldosterone in man. J. Amer. med. Ass. **174**, 234 (1960).
MARUSIC, E. T., MULROW, P. J.: Stimulation of aldosterone biosynthesis in adrenal mitochondria by sodium depletion. J. clin. Invest. **46**, 2101 (1967).
MÜLLER, A. F.: Regulation der Aldosteronsekretion. Verh. Dtsch. Ges. inn. Med., 68. Kongr., S. 599. München: Bergmann 1962.
— MANNING, E. L., MÉGEVAND, R., MORET, P.: Double régulation hémodynamique de l'aldosterone après hémorrhagie ou fistule artério-veineuse. Schweiz. med. Wschr. **94**, 898 (1964).
— MORET, P., MÉGEVAND, R., PATTAY, J., MANNING, E. L.: Régulation de l'aldostérone. Etude expérimentale. Helv. med. Acta **29**, 535 (1962).
MÜLLER, J.: Aldosterone stimulation *in vitro*. III. Site of action of different aldosterone-stimulating substances on steroid biosynthesis. Acta endocr. (Kbh.) **52**, 515 (1966).
— Alterations of aldosterone biosynthesis by rat adrenal tissue due to increased intake of sodium and potassium. Acta endocr. (Kbh.), **58**, 27 (1968).
— Regulation of aldosterone biosynthesis. Monographs on endocrinology, vol. 5. Berlin-Heidelberg-New York: Springer 1971.
— ZIEGLER, W. H.: Stimulation of aldosterone biosynthesis by serotonin. Acta endocr. (Kbh.) **59**, 23 (1968).
MULROW, P. J., GANONG, W. F., CERA, G., KULJIAN, A.: The nature of the aldosterone-stimulating factor in dog kidneys. J. clin. Invest. **41**, 505 (1962).
ROSENKRANTZ, H.: A direct influence of 5-hydroxytryptamine on the adrenal cortex. Endocrinology **64**, 355 (1959).
VEYRAT, R., BRUNNER, H. R., GRANDCHAMP, A., MULLER, A. F.: Inhibition of renin by potassium in man. Acta endocr. (Kbh.) Suppl. **119**, 86 (1967).
VOTH, D., KOHLHARDT, M., TIETZE, K. W.: Zur Frage der renalen Steuerung der Z. glomerulosa der NNR (quantitative morphologische Untersuchungen). Klin. Wschr. **41**, 433 (1963).
WOLF, H. P.: Aldosteron und Aldosteronismus. Klin. Wschr. **42**, 711 (1964).

Control of Cortisol Secretion, CRF (Corticotropin Releasing Factor) (see also Chap. II, p. 35ff.)

DE WIED, D.: The site of the blocking action of dexamethasone on stress-induced pituitary ACTH-release. J. Endocr. **29**, 29 (1964).
EIK-NES, K. B., BROWN, D. M., BRIZZEE, K. R., SMITH, E. L.: Partial purification and properties of a "corticotropin influencing factor" (CIF) from human spinal fluid: an assay method in the trained dog. Endocrinology **69**, 411 (1961).
ESTEP, H. L., ISLAND, D. P., NEY, R. L., LIDDLE, G. W.: Pituitary-adrenal dynamics during surgical stress. J. clin. Endocr. **23**, 419 (1963).
FEKETE, G., GÖRÖG, P.: The inhibitory action of natural and synthetic glucocorticoids on adrenal steroidogenesis at the adrenal level. J. Endocr. **27**, 123 (1963).
FORTIER, C., WARD, D. N.: Limitations of the in vitro pituitary incubation system as an assay for ACTH-releasing activity. Canad. J. Biochem. **36**, 110 (1958).
GUILLEMIN, R.: Hypothalamic factors releasing pituitary hormones. Recent Progr. Hormone Res. **20**, 89 (1964).
— Hypothalamic polypeptides releasing pituitary hormones. Metabolism **13**, 1206 (1964).
HARRIS, G. W.: Central control of pituitary secretion. Handbook of physiology, vol. II. Neurophysiology. Washington: Amer. physiol. Soc. 1960.
HOFMANN, K.: Chemistry and function of polypeptide hormones. Ann. Rev. Biochem. **31**, 213 (1962).
JAMES, V. H. T., LANDON, J.: The investigation of hypothalamic-pituitary-adrenal function. Cambridge: University Press 1968.
KAPPELER, H., SCHWYZER, R.: Synthetic peptides related to the corticotropins (ACTH) and the melanophore stimulating hormones (MSH) possessing corticotropin releasing activity (CRF-activity). Experientia (Basel) **16**, 415 (1960).
— — Synthese eines Heptapeptides mit starker CRF-Wirkung. Helv. chim. Acta **43**, 1453 (1960).
LIDDLE, G. W., ISLAND, D., MEADOR, C. K.: Normal and abnormal regulation of corticotropin secretion in man. Recent Progr. Hormone Res. **18**, 125 (1962).
MARTINI, L., FRASCHINI, F., MOTTA, M.: Neural control of anterior pituitary functions. Recent Progr. Hormone Res. **25**, 439 (1968).
NEY, R. L., SHIMIZU, N., NICHOLSON, W. E., ISLAND, D. P., LIDDLE, G. W.: Correlation of plasma ACTH concentration with adrenocortical response in normal human subjects, surgical patients and patients with Cushing's disease. J. clin. Invest. **42**, 1669 (1963).
PRIVAT DE GARILHE, M., GROSS, C.: On the *in vitro* corticotropin releasing factor (CRF) activity of the heptapeptide: Methionyl-glutaminyl-histidyl-phenylalanyl-arginyl-tryptophyl-glycine. Experientia (Basel) **18**, 92 (1962).
— — PORATH, J., LINDNER, E. B.: Further studies on corticotropin releasing factor (CRF); corticotropin releasing activity of synthetic peptides. Experientia (Basel) **16**, 414 (1960).

RENOLD, A. E., JENKINS, D., FORSHAM, P. H., THORN, G. W.: The use of intravenous ACTH: a study in quantitative adrenocortical stimulation. J. clin. Endocr. **12**, 763 (1952).
RUF, K., STEINER, F. A.: Steroid-sensitive single neurons in rat hypothalamus and midbrain: Identification by microelectrophoresis. Science **156**, 667 (1967).
SCHALLY, A. V., ARIMURA, A., BOWERS, C. Y., KASTIN, A. J., SAVANO, S., REDDING, T. W.: Hypothalamic neurohormones regulating anterior pituitary function. Recent Progr. Hormone Res. **24**, 485 (1968).
— LIPSCOMB, H. S., GUILLEMIN, R.: Isolation and amino acid sequence of α_2-corticotropin-releasing factor (α_2-CRF) from dog pituitary glands. Endocrinology **71**, 164 (1962).
SCHEDL, H. P., CHEN, PH. S., JR., GREENE, G., REDD, D.: The renal clearance of cortisol. J. clin. Endocr. **19**, 1223 (1959).
SMELIK, P. G., SAWYER, C. H.: Effects of implantation of cortisol into the brain stem or pituitary gland on the adrenal response to stress in the rabbit. Acta endocr. (Kbh.) **41**, 561 (1962).
YATES, F. E.: Physiological control of adrenal cortical hormone secretion. In: The adrenal cortex (A. B. EISENSTEIN, ed.), p. 133. Boston: Little, Brown & Co. 1967.
— URQUHART, J.: Control of plasma concentrations of adrenocortical hormones. Physiol. Rev. **42**, 359 (1962).

Circadian Rhythm of Adrenocortical Secretion

COHEN, M., STIEFEL, M., REDDY, W. J., LAIDLAW, J. C.: The secretion and disposition of cortisol during pregnancy. J. clin. Endocr. **18**, 1076 (1958).
DI RAIMONDO, V. C., FORSHAM, P. H.: Some clinical implications of the spontaneous diurnal variation in adrenal cortical activity. Amer. J. Med. **21**, 321 (1956).
DOE, R. P., FLINK, E. B., GOODSELL, M. G.: Relationship of diurnal variation in 17-hydroxycorticosteroid levels in blood and urine to eosinophils and electrolyte excretion. J. clin. Endocr. **16**, 196 (1956).
EIK-NES, K. B., CLARK, L. D.: Diurnal variation of plasma 17-hydroxycorticosteroids in subjects suffering from severe brain damage. J. clin. Endocr. **18**, 764 (1958).
HALBERG, F., PETERSON, R. E., SILBER, R. H.: Phase relations of 24 hour-periodicities in blood corticosterone, mitoses in cortical adrenal parenchyma and total body activity. Endocrinology **6**, 222 (1959).
KRIEGER, D. T.: Diurnal pattern of plasma 17-hydroxycorticosteroids in pretectal and temporal lobe disease. J. clin. Endocr. **21**, 695 (1961).
MIGEON, C. J., TYLER, F. H., MAHONEY, J. P., FLORENTIN, A. A., CASTLE, H., BLISS, L. E., SAMUELS, L. T.: The diurnal variation of plasma levels and urinary excretion of 17-hydroxycorticosteroids in normal subjects, night workers and blind subjects. J. clin. Endocr. **16**, 622 (1956).
ORTH, D. N., ISLAND, D. P., LIDDLE, G. W.: Experimental alteration of the circadian system in plasma cortisol (17 OHCS) concentration in man. J. clin. Endocr. **27**, 549 (1967).
PERKOFF, G. T., EIK-NES, K., NUGENT, C. A., FRED, H. L., NIMER, R. A., RUSK, L., SAMUELS, L. T., TYLER, F. H.: Studies of the diurnal variation of plasma 17-hydroxycorticosteroids in man. J. clin. Endocr. **19**, 432 (1959).

Effects of Adrenocortical Hormones

Reviews

NOWAKOWSKI, H.: Stoffwechselwirkungen der Steroidhormone. 2. Symposion der Dtsch. Ges. Endokrinol. Berlin-Göttingen-Heidelberg: Springer 1955.
THORN, G. W., u. a.: Pharmacological aspects of adrenocortical steroids and ACTH in man. New Engl. J. Med. **248**, 232 (1953).

Aldosterone and Deoxycorticosterone

AUGUST, J. T., NELSON, D. H.: Adjustment to aldosterone or DOCA induced sodium retention in patients with Addisons disease. J. clin. Invest. **38**, 1964 (1959).
— — THORN, G. W.: Aldosterone. New Engl. J. Med. **259**, 917, 967 (1958).
— — — Response of normal subjects to large amounts of aldosterone. J. clin. Invest. **37**, 1549 (1958).
BRICKER, N. S.: The control of sodium excretion with normal and reduced nephron populations. The preeminence of third factor. Amer. J. Med. **43**, 313 (1967).
CRABBÉ, J.: Stimulation of active sodium transport by the isolated toad bladder with aldosterone *in vitro*. J. clin. Invest. **40**, 2103 (1961).
DÖLLE, W.: Die Regulation der renalen Natriumausscheidung durch den sog. „dritten Faktor". Internist (Berl.) **9**, 323 (1968).
EDELMAN, I. S., FIMOGNARI, G. M.: On the biochemical mechanism of action of aldosterone. Recent Progr. Hormone Res. **24**, 1 (1968).
Editorial: The fate of sodium in the renal tubules. New Engl. J. Med. **279**, 381 (1968).
FANESTIL, D. D., EDELMAN, I. D.: On the mechanism of action of aldosterone on sodium transport: Effects of inhibitors of RNA and of protein synthesis. Fed. Proc. **25**, 912 (1966).
FREAZIER, H. S.: Renal regulation of sodium balance. New Engl. J. Med. **279**, 868 (1968).
GAUNT, R., CHART, J. J.: Mineralocorticoid action of adrenocortical hormones. In: Handbuch der experimentellen Pharmakologie, Bd. 14,1. The adrenocortical hormones. Berlin-Göttingen-Heidelberg: Springer 1962.
— RENZI, A. A., CHART, J. J.: Aldosterone — a review. J. clin. Endocr. **15**, 621 (1955).
GROSS, F.: Extrarenale Wirkungen von Aldosteron. Dtsch. med. Wschr. **86**, 1989 (1961).
HIERHOLZER, K., WIEDERHOLT, M., STOLTE, H.: Hemmung der Natriumresorption im proximalen und distalen Konvolut adrenalektomierter Ratten. Pflügers Arch. ges. Physiol. **291**, 43 (1966).
HORTON, R., BIGLIERI, E. G.: Effect of aldosterone on the metabolism of magnesium. J. clin. Endocr. **22**, 1187 (1962).
JOHNSTON, C. I., DAVIS, J. O., HOWARDS, S. S., WRIGHT, F. S.: Cross-circulation experiments on the mechanism of the naturiuresis during saline loading in the dog. Circulat. Res. **20**, 1 (1967).
KASSIRER, J. P., APPLETON, F. M., CHAZAN, J. A., SCHWARTZ, W. B.: Aldosterone in metabolic alkalosis. J. clin. Invest. **46**, 1558 (1967).
LEAF, A.: Ion transport by the isolated bladder of the toad. Résumés des communications. 3. Congr. Intern. Biochim **12—4**, p. 107 (1955).
LICHARDUS, B., PEARCE, J. W.: Evidence for a humoral natriuretic factor released by blood volume expansion. Nature (Lond.) **209**, 407 (1966).
LUETSCHER, J. A., CURTIS, R. H.: Relationship of aldosterone in urine to sodium balance and to some other endocrine functions. J. clin. Invest. **34**, 951 (1955).
MACH, R. S., FABRE, J., DUCKERT, A., BORTH, R., DUCOMMUN, P.: Action clinique et métabolisme de l'aldostérone (Electrocortine). Schweiz. med. Wschr. **84**, 407 (1954).
— MÜLLER, A. F.: Physiopathologie et clinique de l'aldostérone. Schweiz. med. Wschr. **89**, 997 (1959).
MARTINEZ-MALDONALDO, M., KURTZMAN, N. A., RECTOR, F. C., JR., SELDIN, D. W.: Evidence for a hormonal inhibitor of proximal tubular reabsorption. J. clin. Invest. **46**, 1091 (1967).

MÜLLER, A. F., O'CONNOR, C. M.: Aldosterone, an international symposium. London: A. Churchill 1958.
MULROW, P. J.: Metabolic effects of adrenal mineralocorticoid hormones. In: The adrenal cortex (A. B. EISENSTEIN, ed.), p. 293. Boston: Little, Brown & Co. 1967.
NAYLER, W. G.: The inotropic action of d-aldosterone on papillary muscles isolated from monkeys: J. Pharmacol. **148**, 215 (1965).
NOWAKOWSKI, H.: Aldosteron. 9. Symposion der Dtsch. Ges. f. Endokrinologie. Berlin-Göttingen-Heidelberg: Springer 1963.
PORTER, G. A., BOGOROCH, R., EDELMAN, I. S.: On the mechanism of action of aldosterone on sodium transport: The role of RNA synthesis. Proc. nat. Acad. Sci. (Wash.) **52**, 1326 (1964).
— EDELMAN, I. S.: The action of aldosterone and related corticosteroids on sodium transport across the toad bladder. J. clin. Invest. **43**, 611 (1964).
ROSS, E. J.: Aldosterone in clinical and experimental medicine. Springfield: Ch. C. Thomas 1959.
— REDDY, W. J., RIVERA, A., THORN, G. W.: Effects of intravenous infusions of dl-aldosterone acetate on sodium and potassium excretion in man. J. clin. Endocr. **19**, 289 (1959).
SHARP, G. W. G., LEAF, A.: Mechanism of action of aldosterone. Physiol. Rev. **46**, 593 (1966).
SIMPSON, S. A., TAIT, J. F.: Recent progress in methods of isolation, chemistry and physiology of aldosterone. Recent Progr. Hormone Res. **11**, 183 (1955).
TANZ, R. D.: Studies on the inotropic action of aldosterone on isolated cardiac tissue preparations; including the effects of pH, ouabain and SC-8109. J. Pharmacol. **135**, 71 (1962).
WIEDERHOLT, M., HIERHOLZER, K., BRECHT, J. P.: Die Wirkung von Aldosteron und Cortison auf den tubulären Na-Transport adrenalektomierter Ratten. Pflügers Arch. ges. Physiol. **283**, 71 (1965).
WILBRANDT, W.: Die Bedeutung der Corticosteroide für Ionentransporte. Schweiz. med. Wschr. **89**, 363 (1959).

Aldosterone Antagonists

BARTTER, F. C.: The clinical use of aldosterone antagonists. Springfield: Ch. C. Thomas 1960.
BLEDSOE, T., ISLAND, D. P., RIONDEL, A. M., LIDDLE, G. W.: Modification of aldosterone secretion and electrolyte excretion in man by a chemical inhibitor of 18-oxidation. J. clin. Endocr. **24**, 740 (1964).
BUCHBORN, E., KOCZOREK, K. H. R.: Aldosteronantagonisten und ihre klinische Anwendung. Verh. Dtsch. Ges. inn. Med., 68. Kongr., S. 649, 1962.
DESAULLES, P. A.: Preliminary note on certain pharmacological properties of 3β,16α-Dihydroxyallopregnan-20-on. Experentia (Basel) **15**, 301 (1959).
KLEIN, R., TAYLOR, P., PAPADOLOS, C., LARON, Z., KEELE, D., FORTUNATO, J., BYERS, C., BILLINGS, C.: Sodium-losing material in human urine. Proc. Soc. exp. Biol. (N.Y.) **98**, 863 (1958).
LIDDLE, G. W.: Specific and non-specific inhibition of mineralocorticoid activity. Metabolism **10**, 1021 (1961).
MÜLLER, A. F.: Die Aldosteron-Antagonisten. Dtsch. med. Wschr. **86**, 951 (1961).
NEHER, R., DESAULLES, P., VISCHER, E., WIELAND, P., WETTSTEIN, A.: Isolierung, Konstitution und Synthese eines neuen Steroides aus Nebennieren. Helv. chim. Acta **41**, 1667 (1958).
— MEYSTRE, CH., WETTSTEIN, A.: Neue 16α-Hydroxysteroide aus menschlichem Urin und aus Schweine-Nebennieren. Isolierung, Konstitution, Synthese. Helv. chim. Acta **42**, 132 (1959).
SALASSA, R. M., MATTOX, V. R., POWER, M. H.: Effect of an aldosterone antagonist on sodium and potassium excretion in primary hyperaldosteronism. J. clin. Endocr. **18**, 787 (1958).
SCHLATTMANN, R. J. A. F. M., JANSEN, A. P., PRENEN, H., KORST, J. K. VAN DER, MAJOOR, C. L. H.: The natriuretic and aldosterone-suppressive action of heparin and some related polysulfated polysaccharides. J. clin. Endocr. **24**, 35 (1964).

Cortisol and Cortisone

ASBOE-HANSEN, G.: Hormonal effects on connective tissue. Physiol. Rev. **38**, 446 (1958).
BECK, I. T., FLETCHER, H. W., MCKENNA, R. D., GRIFF, H.: Effect of small and massive doses of prednisone on gastric secretory activity. Gastroenterology **38**, 740 (1960).
BEISEL, W. R., RAPOPORT, M. I.: Inter-relations between adrenocortical functions and infectious illness. New Engl. J. Med. **280**, 541, 596 (1969).
BICKEL, G., SECRÉTAN, PH.: Cortisone et gestations. Bull. schweiz. Akad. med. Wiss. **11**, 125 (1955).
BODO, R. C. DE, STEELE, R., ALZSZULER, N., DUNN, A., BISHOP, J. S.: On the hormonal regulation of carbohydrate metabolism; studies with C^{14}-glucose. Recent Progr. Hormone Res. **19**, 445 (1963).
DAVIS, R. A., BROOKS, F. P.: Gastric secretion, continously recorded blood sugar and plasma steroids after insulin. Amer. J. Physiol. **204**, 143 (1963).
DOUGHERTY, T. F., BERLINER, D. L., BERLINER, M. L.: Corticosteroid-tissue interactions. Metabolism **10**, 996 (1961).
FAJANS, S. S.: Some metabolic actions of corticosteroids. Metabolism **10**, 951 (1961).
FROESCH, E. R., WINEGRAD, A. I., RENOLD, A. E., THORN, G. W.: Mechanism of the glucosuria produced by the administration of steroids with glucocorticoid activity. J. clin. Invest. **37**, 524 (1958).
GORDON, A. S.: Endocrine influences upon the formed elements of blood and blood-forming organs. Recent Progr. Hormone Res. **10**, 339 (1954).
GRAY, S. J., RAMSEY, C. G.: Adrenal influences upon the stomach and the gastric responses to stress. Recent Progr. Hormone Res. **13**, 583 (1957).
HASTINGS, A. B., RENOLD, A. E., TENG, CH. T.: Effects of ions and hormones on carbohydrate metabolism. Recent Progr. Hormone Res. **11**, 381 (1955).
JEANRENAUD, B.: Fonctions et régulation hormonale du tissu adipeux. Helv. med. Acta **30**, 1 (1963).
— RENOLD, A. E.: Studies on rat adipose tissue *in vitro*. J. biol. Chem. **235**, 2217 (1960).
JERVELL, K. F.: Early effects of glucoçorticoids on ribonucleic acid and protein metabolism in rat liver. Acta endocr. (Kbh.) **44**, Suppl. 88 (1963).
LAAKE, H.: The action of corticosteroids on the renal reabsorption of calcium. Acta endocr. (Kbh.) **60**, 34 (1960).
LEVINE, R., GOLDSTEIN, M. S.: Glucocorticoid action of adrenocortical hormones. In: Handbuch d. exp. Pharmakologie, Bd. XIV/2. The adrenocortical hormones, ed by H. W. DEANE u. B. I. RUBIN. Berlin-Göttingen-Heidelberg: Springer 1964.
MAKMAN, H. M., NAKAGAWA, S., WHITE, A.: Studies of the mode of action of adrenal steroids on lymphocytes. Recent Progr. Hormone Res. **23**, 195 (1967).
MARGULIS, R. R., HODGKINSON, C. P., HOWARD, P. J., GORDON, E. I.: Effects of administration of adrenocorticotropic hormone and cortisone during pregnancy upon mothers, developing fetuses and infants. J. clin. Endocr. **14**, 779 (1954).
RAY, P. D.: Adrenal glucocorticoids and gluconeogenesis. In: Functions of the adrenal cortex, vol. II, ed. by K. W. MCKERNS. Amsterdam: North Holland Publ. Comp. 1968.

REID, N.C.R.W., HACKETT, R.M., WELBOURN, R.B.: The influence of cortisone on the parietal cell population of the stomach in the dog. Gut **2**, 119 (1961).
RENOLD, A.E., FROESCH, E.R., REARDEN, J.B., FINKENSTAEDT, J.T.: Effects of hydrocortisone on carbohydrate metabolism of a patient with renal glycosuria (De Toni Fanconi Syndrom). (Abstr.) J. clin. Invest. **34**, 960 (1955).
ROTHSCHILD, M.A., SCHREIBER, S.S., ORATZ, M., MCGEE, H.L.: The effects of adrenocortical hormones on albumin metabolism, studies with albumin I^{131}. J. clin. Endocr. **18**, 1229 (1958).
SIRCUS, W., HUSTON, C.J.W., PRESTRAW, R.M., BASSOE, H., HARKNESS, R.A.: Studies in dogs on the biphasic nature of the gastric secretory response to hypoglycaemia and other stimuli with special reference to the role of the adrenals. Gut **4**, 42 (1963).
SPIRO, H.M., MILLER, S.S.: Clinical and physiologic implications of the steroid-induced peptic ulcer. New Engl. J. Med. **263**, 286 (1960).
STUDER, A.: Zur Frage der Angriffsorte vom Compound F (Cortison). Z. ges. exp. Med. **121**, 287 (1953).
THORN, G.W.: The effects of glucocorticoids upon growth and development. Schweiz. med. Wschr. **100**, 1625 (1970).
— RENOLD, A.E., CAHILL, G.F., JR.: The adrenal and diabetes. Some interactions and interrelations. Diabetes **8**, 337 (1959).
— — WINEGRAD, A.I.: Some effects of adrenal cortical hormones on intermediary metabolism. Brit. med. J. **1957 II**, 1009.
WALSER, M., ROBINSON, B.H.B., BUCKETT, J.W.: The hypercalcemia of adrenal insufficiency. J. clin. Invest. **42**, 456 (1963).
WEBER, G.: Action of glucocorticoid hormone at the molecular level. In: Functions of the adrenal cortex, ed. by K.W. MCKERNS. Amsterdam: North Holland Publ. Comp. 1968.
WEISSMANN, G.: The effects of corticosteroids, especially on lysosomes and biomembranes. In: W. MÜLLER, H.G. HARWERTH, K. FEHR (eds.), Rheumatoid arthritis, Colloquia Geigy, p. 577. London-New York: Academic Press 1971.
— THOMAS, L.: The effects of corticosteroids upon connective tissue and lysosomes. Recent Progr. Hormone Res. **20**, 215 (1964).
WILBER, J.F., UTIGER, R.D.: The effect of glucocorticoids on thyrotropin secretion. J. clin. Invest. **48**, 2096 (1969).

Adrenal Androgens

COHN, G.L., BONDY, P.K., CASTIGLIONE, C.: Studies of pyrogenic steroids. I. Separation, identification and measurement of unconjugated dehydroepiandrosterone, etiocholanolone and androsterone in human plasma. J. clin. Invest. **40**, 400 (1961).
— MULROW, P.J.: Androgen release and synthesis *in vitro* by human adult adrenal glands. J. clin. Invest. **42**, 64 (1963).
HOWARD, E., MIGEON, C.: Sex hormone secretion by the adrenal cortex. In: Handbuch der experimentellen Pharmakologie, Bd. 14, 1, The adrenocortical hormones. Berlin-Göttingen-Heidelberg: Springer 1962.
KAPPAS, A.: Studies in endocrine pharmocology: Biologic actions of some natural steroids on the liver. New Engl. J. Med. **278**, 378 (1968).
— PALMER, R.H., GLICKMANN, P.B.: Steroid fever. Amer. J. Med. **31**, 167 (1961).
MIGEON, C.J., KELLER, A.R., LAWRENCE, B., SHEPARD, TH.H.: Dehydroepiandrosterone and androsterone levels in human plasma. Effect of age and sex. Day-to-day and diurnal variations. J. clin. Endocr. **17**, 1051 (1957).
SHULMAN, J.A., HERRMANN, W.L., PETERSDORF, R.G.: Experimental etiocholanolone fever. J. clin. Endocr. **24**, 1136 (1964).
TAMM, J.: Steroidfieber. Dtsch. med. Wschr. **88**, 1794 (1963).
WIELE, R. VAN DE, LIEBERMANN, S.: The metabolism of Dehydroisoandrosterone. In: Biological activities of steroids in relation to cancer, ed. by G. PINCUS and E.P. VOLLMER. New York: Academic Press 1960.

Synthetic Steroids (cf. Chap. XX)

BOLAND, E.W.: Antirheumatic potency of chemically modified adrenocortical steroids. Amer. J. Med. **31**, 581 (1961).
— Clinical comparison of the newer anti-inflamatory corticosteroids. Ann. rheum. Dis. **21**, 176 (1962).
DRILL, V.A., RIEGEL, B.: Structural and hormonal activity of some new steroids. Recent Progr. Hormone Res. **14**, 29 (1958).
KAISER, H.: Cortisonderivate in Klinik und Praxis, 5. Aufl. Stuttgart: Thieme 1968.
KENDALL, J.W., JR., LIDDLE, G.W., FEDERSPIEL, CH.F., CORNFIELD, J.: Dissociation of corticotropin-suppressing activity from the eosinopenic and hyperglycemic activities of corticosteroid analogues. J. clin. Invest. **42**, 396 (1963).
LIDDLE, G.W., FOX, M.: Structure-function relationships of anti-inflammatory steroids. In: L.C. MILLS and J.H. MOYER, Inflammation and diseases of connective tissue. Philadelphia: Saunders 1961.
SIEGENTHALER, W., SIEGENTHALER, G., MANN, M., BONER, A., CHRISTEN, PH.: Die Corticosteroidtherapie in der inneren Medizin. Praxis **14**, 490 (1964).
THORN, G.W.: Clinical considerations in the use of corticosteroids. New Engl. J. Med. **274**, 775 (1966).

The General Adaptation Syndrome

BAUER, W., CLARK, W.S.: The relationship of the adaptation concept to the connective tissue disease. Recent Progr. Hormone Res. **8**, 217 (1953).
DICZFALUSY, E., CASSMER, O., ULMACK, R.: Assessment of the functional reserve capacity of the adrenal cortex in healthy subjects following exhaustive exercise. J. clin. Endocr. **22**, 78 (1962).
HEDINGER, CHR.: Anpassung, Anpassungskrankheiten und Nebennieren. Schweiz. med. Wschr. **1954**, 465.
HILL, S.R., u.a.: Studies on adrenocortical and psychological response to stress in man. Arch. intern. Med. **97**, 269 (1956).
INGLE, D.J.: Permissibility of hormone action. A review. Acta endocr. (Kbh.) **17**, 172 (1954).
— BAKER, B.L.: A consideration of the relationships of experimentally produced and naturally occurring pathologic changes in the rat to the adaptation diseases. Recent Progr. Hormone Res. **8**, 143 (1953).
LABHART, A.: Die Bedeutung des Adaptationssyndroms für die Klinik. Regensburg. Jb. ärztl. Fortbild. **7** (1958/59).
NELSON, D.H.: Disorders of ACTH-secretion in man. Metabolism **10**, 894 (1961).
SCHÜPBACH, A.: Sinn und Bedeutung der Hormone. Praxis **1951**, 131.
SELYE, H.: The general adaptation syndrome. J. clin. Endocr. **6**, 117 (1946).
— Stress. Montreal: Acta Inc. 1950.
— Einführung in die Lehre vom Adaptationssyndrom. Stuttgart: Georg Thieme 1953.
— HORAVA, A., HEUSER, G.: Annual reports on stress (1st—5th). Montreal: Acta Inc. 1951—1956.

Hypofunction

Reviews

HOFMANN, F.G., SOBEL, E.H.: Adrenocortical insufficiency In: Handbuch der experimentellen Pharmakologie, Bd. XIV/2. The adrenocortical hormones, ed. by H.W. DEANE and B.L. RUBIN. Berlin-Göttingen-Heidelberg: Springer 1964.

KRÜCK, F.: Morbus Addison. Chronische Nebennierenin-suffizienz. Internist (Berl.) **5**, 12 (1964).
SOFFER, L.J., DORFMAN, R.I., GABRILOVE, J.L.: The human adrenal gland. Philadelphia: Lea & Febiger 1961.
THORN, G.W.: The diagnosis and treatment of adrenal insufficiency, 2nd ed. Springfield: Thomas 1951. Deutsch: Nebenniereninsuffizienz, Diagnose und Behandlung. Bern u. Stuttgart: Huber 1953.

Addison's Disease

Pathological Anatomy, Incidence, Etiology and Pathogenesis

ANDERSON, J.R., BUCHANAN, W.W., GOUDIE, R.B.: Autoimmunity, clinical and experimental. Springfield/Ill.: Thomas 1967.
ANDRADA, J.A., BIGAZZI, P.L., ANDRADA, E., MILGROM, F., WITEBSKY, E.: Serological investigations on Addison's disease. J. Amer. med. Ass. **206**, 1535 (1968).
BACHMANN, R.: Die Nebenniere. In: W.v. MOELLENDORFF u. W. BARGMANN, Handbuch der mikroskopischen Anatomie, Bd. 6, Teil 5, S.1. Berlin-Göttingen-Heidelberg: Springer 1954.
BLIZZARD, R.M., CHEE, D., DAVIS, W.: The incidence of adrenal and other antibodies in the sera of patients with idiopathic adrenal insufficiency (Addison's disease). Clin. exp. Immunol. **2**, 19 (1967).
— KYLE, M.: Studies of the adrenal antigens and antibodies in Addison's disease. J. clin. Invest. **42**, 1653 (1963).
CRISPELL, K.R., PARSON, W., HAMLIN, J., HOLLIFIELD, G.: Addison's disease associated with histoplasmosis. Amer. J. Med. **20**, 23 (1956).
DEL NEGRO, G., WAJCHENBERG, B.L., PEREIRA, V.G., SCHNAIDER, J., ULHVA CINTRA, A.B. DE, MARQUES DE ASSIS, L., PRADO SAMPAIO, S.A.: Addison's disease associated with South American blastomycosis. Ann. intern. Med. **54**, 189 (1961).
FISH, R.G., TAKARO, T., LOVELL, M.: Coexistent Addison's disease and north American blastomycosis. Amer. J. Med. **28**, 152 (1960).
FRIEDMAN, N.B.: The pathology of the adrenal gland in Addison's disease with special reference to adrenocortical contraction. Endocrinology **42**, 181 (1948).
GALLOWAY, J.A., PERLOFF, W.H.: Addison's disease secondary to adrenocortical destruction by metastatic carcinoma of the breast. Amer. J. Med. **28**, 156 (1960).
GSELL, O., UEHLINGER, E.: Tuberkulöser Morbus Addison. Stellung der Nebennierentuberkulose im Ablauf der tuberkulösen Infektion. Beitr. Klin. Tuberk. **83**, 121 (1933).
GUTTMAN, P.H.: Addison's disease. A statistical analysis of 566 cases and a study of the pathology. Arch. Path. **10**, 742 (1930).
IRVINE, W.J., STEWART, A.G., SCARTH, L.: A clinical and immunological study of adrenocortical insufficiency (Addison's disease). Clin. exp. Immunol. **2**, 31 (1967).
Leading article: Autoimmunity in idiopathic Addison's disease. Lancet **1967 I**, 1040.
LIEBEGOTT, G.: Studien zur Orthologie und Pathologie der Nebennieren. Beitr. path. Anat. **109**, 93 (1947).
MASON, A.S., MEADE, T.W., LEE, J.A.H., MORRIS, J.N.: Epidemiological and clinical picture of Addison's disease. Lancet **1968 II**, 744.
MEAD, R.K.: Autoimmune Addison's disease. New Engl. J. Med. **266**, 583 (1962).
PASCHKIS, K.E., RAKOFF, A.E., CANTAROW, A., RUPP, J.J.: Clinical endocrinology, 3rd ed. New York-Evanston-London: Hoeber 1967.
POUSSET, G., TOURNIAIRE, J., MONIER, J.C., BONHOMME, C.: Les auto-anticorps anti-surrénaliens dans l'insuffisance surrénale chronique. Rev. franç. Endocr. clin. **4**, 305 (1968).
SCHMIDT, M.B.: Eine biglanduläre Erkrankung bei M. Addison. Verh. dtsch. path. Ges. **37**, 121 (1926).
SLOPER, J.T.: Pathology of adrenals, thymus and certain other endocrine glands in Addison's disease: Analysis of 37 necropsies. Proc. roy. Soc. Med. **48**, 625 (1955).
STEINER, J.W., LANGER, B., SCHATZ, D.L., VOLPE, R.: Experimental immunologic adrenal injury. J. exp. Med. **112**, 187 (1960).
WUEPPER, K.D., WEGIENKA, L.C., FUDENBERG, H.H.: Immunologic aspects of adrenocortical insufficiency. Amer. J. Med. **46**, 206 (1969).

Clinical Picture, Symptomatology, Diagnostic Procedures

AHMED, A.B., GEORGE, B.C., GONZALES-AUVERT, C.: Increased plasma arginine vasopressin in clinical adrenocortical insufficiency and its inhibition by glucosteroids. J. clin. Invest **46**, 111 (1967).
CARRYER, H.M., SHERRICK, D.W., GASTINEAU, C.F.: Occurence of allergic disease in patients with adrenal cortical hypofunction. J. Amer. med. Ass. **172**, 1356 (1960).
FELLOWS, R.E., BUCHANAN, J.R., PETERSON, R.E., STOKES, P.E.: Chronic primary adrenal insufficiency without hyperpigmentation. New Engl. J. Med. **267**, 215 (1962).
GILL, J.R., JR., GANN, D.S., BARTTER, F.C.: Restoration of diuresis in Addisonian patients by expansion of volume of extracellular fluid. J. clin. Invest. **41**, 1078 (1962).
HALL, T.C., MCCRACKEN, B.H., THORN, G.W.: Skin pigmentation in relation to adrenal cortical function. J. clin. Endocr. **13**, 243 (1953).
HARRIS-JONES, J.N., NIXON, P.G.F.: Familial Addison's disease with spastic paraplegia. J. clin. Endocr. **15**, 739, 1955.
HEDINGER, CHR.: Zur Frage des Morbus Addison ohne Pigmentvermehrung (sog. weißer Morbus Addison). Schweiz. med. Wschr. **1950**, 489.
— Zur Pathologie der Skeletmuskulatur. 2. Mitteilung. Myalgien als führendes Symptom gewisser Fälle von Addisonscher Krankheit. Schweiz. med. Wschr. **1952**, 869.
HENKIN, R.I., BARTTER, F.C.: Studies on olfactory thresholds in normal man and in patients with adrenal cortical insufficiency: the role of adrenal cortical steroids and of serum sodium concentration. J. clin. Invest. **45**, 1631 (1966).
— GILL, J.R., JR., BARTTER, F.C.: Studies on taste thresholds in normal man and in patients with adrenal cortical insufficiency: the role of adrenal cortical steroids and of serum sodium concentration. J. clin. Invest. **42**, 727 (1963).
— MCGLONE, R.E., DALY, R., BARTTER, F.C.: Studies of auditory thresholds in normal man and in patients with adrenal cortical insufficiency: the role of adrenal cortical steroids. J. clin. Invest. **46**, 429 (1967).
— SOLOMON, D.H.: Salt taste threshold in adrenal insufficiency in man. J. clin. Endocr. **22**, 856 (1962).
JARRIS, J.L., JENKINS, D., SOSMAN, M.C., THORN, G.W.: Roentgenologic observations in Addison's disease. Radiology **62**, 16 (1954).
KLEEMAN, C.R., CZACZKES, J.W., CUTLER, R.: Mechanisms of impaired water excretion in adrenal and pituitary insufficiency. IV. Antidiuretic hormone in primary and secondary adrenal insufficiency. J. clin. Invest. **43**, 1641 (1964).
LAIDLAW, J.C., REDDY, W.J., JENKINS, D., HAYDAR, N.A., RENOLD, A.E., THORN, G.W.: Advances in the diagnosis of altered states of adrenocortical function. New Engl. J. Med. **253**, 747 (1955).
LEEKSMA, C.H.W., GRAEFF, J. DE, COCK, J. DE: Hypercalcemia in adrenal insufficiency. Acta med. Scand. **156**, 455 (1957).
LORME, A. DE: Hypercalcämie bei M. Addison. Dissertation, Zürich 1964.

McBrien, D.J., Vaughan Jones, R., Creamer, B.: Steatorrhoea in Addison's disease. Lancet **1963 I**, 25.
Morse, W.I., Criscitiello, M.G., Amador, E., Renold, A.E., Harrison, J.H., Dammin, G.J., Thorn, G.W.: Bilateral adrenalectomy for hypertensive vascular disease. Amer. J. Med. **26**, 315 (1959).
Peart, W.S.: The function of renin and angiotensin. Recent Progr. Hormone Res. **21**, 73 (1965).
Penman, R.W.: Addison's disease in association with spastic paraplegia. Brit. med. J. 402, 1960
Pollen, R.H., Williams, R.H.: Hyperkalemic neuromyopathy in Addison's disease. New Engl. J. Med. **263**, 273 (1960).
Prader, A., Uehlinger, E., Illig, R.: Hypercalcämie bei M. Addison im Kindesalter. Helv. paediat. Acta **14**, 607 (1959).
Renold, A.E., Jenkins, D., Forsham, P.H., Thorn, G.W.: The intravenous use of ACTH. A study in quantitativ adrenocortical stimulation. J. clin. Endocr. **12**, 763 (1952).
Somerville, W., Levine, H.D., Thorn, G.W.: The electrocardiogram in Addison's disease. Medicine (Baltimore) **30**, 43 (1951).
Stoll, W.A.: Die Psychiatrie des M. Addison. Stuttgart: Georg Thieme 1953.
— Psychopathology of hypoadrenocorticism. In: Handbuch der experimentellen Pharmakologie, Bd. 14, Teil 1 ed. by H.W. Deane. Berlin-Göttingen-Heidelberg: Springer 1962.
Symington, Th.: Functional pathology of the human adrenal gland. Edinburgh-London: Livingstone 1969.
Walser, M., Robinson, B.H., Duckett, J.W.: The hypercalcaemia of adrenal insufficiency. J. clin. Invest. **42**, 456 (1963).

Pregnancy in Addison's Disease

Cope, C.L., Black, E.: Hydrocortisone production in late pregnancy, J. Obstet. Gynaec. Brit. Emp. **66**, 404 (1959).
Decourt, J., Robel, P., Michard, J.P.: Evolution d'une grossesse chez une femme ayant subi une surrénalectomie bilaterale totale pour maladie de Cushing. Ann. Endocr. (Paris) **22**, 217 (1961).
Hills, A.G., Venning, E.H., Dohan, F.C., Webster, G.D., Richardson, E.M.: Pregnancy and adrenocortical function: endocrine studies of pregnancy occurring in two adrenal-deficient women. J. clin. Invest. **33**, 1466 (1954).
Migeon, C.J., Bertrand, J., Wall, P.E.: Physiological disposition of 4-C^{14}-Cortisol during late pregnancy. J. clin. Invest. **36**, 1350 (1957).
— — — Stempfel, R.S., Prystowsky, H.: Metabolism and placental transmission of cortisol during pregnancy, near term. Ciba Coll. **11**, 338 (1957).
Neher, R., Stark, G.: Nachweis von Corticosteroiden in menschlicher Placenta und Isolierung von 16α-Hydroxytestosteron. Experientia (Basel) **17**, 510 (1961).
Osler, M.: Addison's disease and pregnancy. Acta endocr. (Kbh.) **41**, 67 (1962).
Sims, E.A.H., Meeker, C.I., Gray, M.J., Watanabe, M., Solomon, S.: The secretion of aldosterone in normal pregnancy and in preeclampsia. In: Aldosterone, ed. by E.E. Baulieu and P. Robel, p. 499. Oxford 1964.

Special Forms of Adrenocortical Failure

Acute Insufficiency due to Hemorrhagic Infarction

Bamatter, F.: Fulminante Meningokokkensepsis. Zur Ätiologie des Syndroms von Waterhouse-Friderichsen. Jb. Kinderheilk. **142**, 127 (1934).
Berte, S.J.: Spontaneous adrenal hemorrhage in the adult. Review and report of 2 cases. Ann. intern. Med. **38**, 28 (1953).
Clavadetscher, P., Zellweger, J.P., Hedinger, Chr.: "Idiopathische" Nebennierenblutungen. Schweiz. med. Wschr. **100**, 576 (1970).
Ferguson, J.H., Chapman, O.D.: Fulminating meningococcus infections and the so called Waterhouse-Friderichsen-Syndrome. Amer. J. Path. **24**, 763 (1948).
Fox, B.: Adrenal haemorrhage and necrosis resulting from abdominal operations. Lancet **1969 I**, 600.
Friderichsen, C.: Waterhouse-Friderichsen-Syndrome. Acta endocr. (Kbh.) **18**, 482 (1955).
Harper, J.R., Ginn, W.M., Jr., Taylor, W.J.: Bilateral adrenal hemorrhage — A complication of anticoagulant therapy. Amer. J. Med. **32**, 984 (1962).
Klingenberg, A.: Zur massiven Nebennierenblutung des Neugeborenen. Schweiz. med. Wschr. **100**, 417 (1970).
Merz, W., Auf der Maur, A.: Nebennierenapoplexie bei Heparinverabreichung. Schweiz. med. Wschr. **82**, 590 (1952).
Stuber, H.W., Hitzig, W.H.: Zur Pathogenese und Therapie des Waterhouse-Friderichsen-Syndroms. Beziehungen zum Sanarelli-Shwartzman-Phänomen. Schweiz. med. Wschr. **91**, 1612 (1961).
Tönz, O., Aufdermauer, F.: Erfolgreiche Behandlung eines Waterhouse-Friderichsen-Syndroms mit Heparin. Schweiz. med. Wschr. **97**, 1611 (1967).

Congenital Adrenocortical Insufficiency due to Lipoidhyperplasia of the Adrenals

Camacho, A.M., Kowarski, A., Migeon, C.J., Brough, A.J.: Congenital adrenal hyperplasia due to a deficiency of one of the enzymes involved in the biosynthesis of pregnenolone. J. clin. Endocr. **28**, 153 (1968).
Gurtner, H.P.: Pseudohermaphroditismus masculinus und kongenitale Nebennierenrindenhyperplasie. Virchows Arch. path. Anat. **326**, 409 (1955).
O'Doherty, N.J.: Lipoid adrenal hyperplasia. Guy's Hosp. Rep. **113**, 368 (1964).
Prader, A., Anders, G.J.P.A.: Zur Genetik der kongenitalen Lipoidhyperplasie der Nebennieren. Helv. paediat. Acta **17**, 285 (1962).
— Gurtner, H.P.: Das Syndrom des Pseudohermaphroditismus masculinus bei kongenitaler Nebennierenrinden-Hyperplasie ohne Androgenüberproduktion (adrenaler Pseudohermaphroditismus masculinus). Helv. paediat. Acta **10**, 397 (1955).
— Siebenmann, R.E.: Kongenitale Lipoidhyperplasie der Nebennierenrinde. Helv. paediat. Acta **12**, 569 (1957).
Siebenmann, R.E.: Die kongenitale Lipoidhyperplasie der Nebennierenrinde bei Nebennierenrindeninsuffizienz. Schweiz. Z. Path. **20**, 77 (1957).

Latent Addison's Disease

Abu Haydar, N., Marc, J.R.St., Reddy, W.J., Laidlaw, J.C., Thorn, G.W.: Adrenocortical insufficiency with normal basal levels of urinary 17-hydroxycorticoids: Diagnostic implications. J. clin. Endocr. **18**, 121 (1958).
Nordin, B.E.C.: Addison's disease with partial recovery. Proc. roy. Soc. Med. **48**, 1024 (1955).
Smith, H.: Compensated adrenocortical failure. Lancet **1963 I**, 1077.

Addison's Disease and Cerebral Sclerosis

Fanconi, A., Prader, A., Isler, W., Luethy, F., Siebenmann, R.: Morbus Addison mit Hirnsklerose im Kindesalter. Helv. paed. Acta **18**, 480 (1963).

HOEFNAGEL, D., NOORT, S. VAN DEN, INGBAR, S. H.: Diffuse cerebral sclerosis with endocrine abnormalities in young males. Brain **85**, 553 (1962).

Selective Failure of Single Groups of Hormones

BOTTICELLI, J. T., LANGE, R. L., KELLY, O. A.: Postural hypotension with decreased blood volume and impaired aldosterone response. Amer. J. Med. **37**, 147 (1964).
HILLS, A. G.: Selective hypoaldosteronism. Amer. J. Med. **26**, 502 (1959).
HUDSON, J. B., CHOBANIAN, A. V., RELMAN, A. S.: Hypoaldosteronism. New Engl. J. Med. **257**, 529 (1957).
KRIEGER, D. T., KRIEGER, H. P.: Aldosterone excretion in pretectal disease. J. clin. Endocr. **24**, 1055 (1964).
LAMBREW, C. T., CARVER, S. T., PETERSON, R. E., HORWITH, M.: Hypoaldosteronism as a cause of hyperkalemia and syncopal attacks in a patient with complete heart block. Amer. J. Med. **31**, 81 (1961).
ROYER, P.: L'hypoaldostéronisme congénital. Rev. franç. Étud. clin. biol. **12**, 111 (1967).
SKANSE, B., HÖKFELT, B.: Hypoaldosteronism with otherwise intact adrenocortical function, resulting in a characteristic clinical entity. Acta endocr. (Kbh.) **28**, 29 (1958).
SLATON, P. E., BIGLIERI, E. G.: Reduced aldosterone excretion in patients with autonomic insufficiency. J. clin. Endocr. **27**, 37 (1967).
STEMPFEL, R. S., JR., ENGEL, F. L.: A congenital familial syndrome of adrenocortical insufficiency without hypoaldosteronism. J. Pediat. **57**, 443 (1960).
ULICK, S., GAUTIER, E., VETTER, K. K., MARKELLO, J. R., YAFFE, S., LOSE, C. U.: An aldosterone biosynthetic defect in a salt-losing disorder. J. clin. Endocr. **24**, 669 (1964).
VISSER, H. K. A., COST, W. S.: A new hereditary defect in the biosynthesis of aldosterone. Acta endocr. (Kbh.) **47**, 589 (1964).
WILSON, I. D., GOETZ, F. C.: Selective hypoaldosteronism as after prolonged heparin administration. Amer. J. med. **36**, 635 (1964).

Secondary Insufficiency

BAYLISS, R. I. S.: Surgical collapse during and after corticosteroid therapy. Brit. med. J. **1958II**, 935.
BETHGE, H.: Die steroidinduzierte Nebennierenrindenunterfunktion. Pathogenese, Klinik, Diagnostik, Prophylaxe und Therapie. Klin. Wschr. **48**, 317 (1970).
CARREON, G. G., CANARY, J. J., MEYER, R. J., KYLE, L. H.: Adrenocortical function after long term corticoid therapy. J. Lab. clin. Med. **56**, 235 (1960).
CHAKMAKJIAN, Z. H., NELSON, D. H., BETHUNE, J. E.: Adrenocortical failor in hypopituitarism. J. clin. Endocr. **28**, 259 (1968).
FRAZER, CH. G., PREUSS, F. S., BIGFORD, W. D.: Adrenal atrophy and irreversible shock, associated with cortisone therapy. J. Amer. med. Ass. **149**, 1542 (1952).
GRABER, A. L., NEY, R. L., NICHOLSON, W. E., ISLAND, D. P., LIDDLE, G. W.: Natural history of pituitary-adrenal recovery following long-term suppression with corticosteroids. J. clin. Endocr. **25**, 11 (1965).
HENNEMAN, PH. H., WANG, D. M. K., IRWIN, J. W., BURRAGE, W. S.: Syndrome following abrupt cessation of prolonged cortisone therapy. J. Amer. med. Ass. **158**, 384 (1955).
HOLUB, D. A., JAILER, J. W., KITAY, J. I., FRANTZ, A. G.: Direct and indirect estimations of pituitary adrenocorticotropin reserves in man following adrenal steroid therapy. J. clin. Endocr. **19**, 1540 (1959).
— KITAY, J. I., JAILER, J. W.: Effects of exogenous adrenocorticotropic hormone (ACTH) upon pituitary ACTH-concentration after prolonged cortisone treatment and stress. J. clin. Invest. **38**, 291 (1959).
— WALLACE, E. Z., JAILER, J. W.: Pituitary adrenocorticotropin (ACTH) reserve in man following prolonged ACTH therapy. J. clin. Endocr. **20**, 1294 (1960).
JASANI, M. K., BOYLE, J. A., GREIG, W. R., DALAKOS, T. G., BROWNING, M. C. K., THOMPSON, A., BUCHANAN, W. W.: Corticosteroid-induced suppression of the hypothalamo-pituitary-adrenal axis: Observations on patients given oral corticosteroids for rheumatoid arthritis. Quart. J. Med. **36**, 261 (1967).
LEWIS, L., ROBINSON, R. F., YEE, J., HACKER, L. A., EISEN, G.: Fatal adrenal cortical insufficiency precipitated by surgery during continuous cortisone treatment. Ann. intern. Med. **39**, 116 (1953).
LABHART, A., MÜLLER, J., MAYOR, G.: Der chirurgische Eingriff unter hochdosierter Cortison-Therapie, bei Cushing-Syndrom und bei primärer oder sekundärer Nebennierenrinden-Insuffizienz. In: Chirurgische Operationslehre. Bd. I, 9. Founded BREITNER, ed. by ZUKSCHWERDT und KRAUS. München-Berlin-Wien: Urban u. Schwarzenberg 1969.
ODELL, W. D., GREEN, G. M., WILLIAMS, R. H.: Hypoadrenotropism: The isolated deficiency of adrenotropic hormone. J. clin. Endocr. **20**, 1017 (1960).
SAMPSON, PH. A., WINSTONE, N. E., BROOKE, B. N.: Adrenal function in surgical patients after steroid therapy. Lancet **1962II**, 321.
SAVAGE, O., COPEMAN, W. S. C., CHAPMAN, L., WELLS, M. V., TREADWELL, B. L. S.: Pituitary and adrenal hormones in rheumatoid arthritis. Lancet **1962I**, 232.
SALASSA, R. M., BENNETT, W. A., KEATING, F. R., JR., SPRAGUE, R. G.: Postoperative adrenal cortical insufficiency: occurence in patients previously treated with cortisone. J. Amer. med. Ass. **152**, 1509 (1953).
WINSTONE, N. E., BROOKE, B. N.: Effects of steroid treatment on patients undergoing operation. Lancet **1961I**, 973.

Congenital Adrenal Hypoplasia

ANGEVINE, D. M.: Pathologic anatomy of hypophysis and adrenals in anencephaly. Arch. Path. **26**, 507 (1938).
BLIZZARD, R. M., ALBERTS, M.: Hypopituitarism, hypoadrenalism and hypogonadism in the newborn infant. J. Pediat. **48**, 782 (1956).
GEPPERT, L. J., SPENCER, W. A., RICHMOND, A. M.: Adrenal insufficiency in infancy. J. Pediat. **37**, 1 (1950).
KOETTGEN, U.: Pylorusstenose und Nebenniereninsuffizienz. Mschr. Kinderheilk. **10**, 24 (1953).

Therapy of Adrenocortical Failure

HILLS, A. G., ZINTEL, H. A., PARSONS, D. W.: Observations of human adrenal cortical deficiency. With special reference to replacement therapy with cortisone. Amer. J. Med. **21**, 358 (1956).
KUPPERMAN, H. S., EPSTEIN, J. A.: Oral therapy of adrenal cortical hypofunction. Use of combined fludrocortisone acetate and hydrocortisone. J. Amer. med. Ass. **159**, 1447 (1955).
LEDINGHAM, J. G. G., MARTIN, F. I. R., MOXHAM, A., HURTER, R., NABARRO, J. D. N.: The metabolic effects of aldosterone given by mouth. Lancet **1961I**, 630.
LIPSETT, M. B., PEARSON, O. H.: Pathophysiology and treatment of adrenal crisis. New Engl. J. Med. **254**, 511 (1956).
MELBY, J. C., CYR, M. ST.: Comparative studies on absorption and metabolic disposal of water-soluble corticosteroid-esters. Metabolism **10**, 75 (1961).
NELSON, D. H., COOPER, C. E.: Relative effectiveness of d-aldosterone given by oral and buccal routes. J. clin. Endocr. **22**, 547 (1962).
ROSS, E. J.: Human assay of electrolyte active steroids and their antagonists. Clin. Sci. **23**, 197 (1962).

SANFORD, J. P., FAVOUR, C. B.: The interrelationship between Addison's disease and active tuberculosis: A review of 125 cases of Addison's disease. Ann. intern. Med. **45**, 56 (1956).

Hyperfunction

Aldosterone Excess

Reviews

Aldosteron. 6. Symposion der Dtsch. Ges. f. Endokrinologie, ed. by H. NOWAKOWSKI. Berlin-Göttingen-Heidelberg: Springer 1963.

BIGLIERI, E. G., SLATON, P. E., SCHAMBELAN, M., KRONFIELD, S. J.: Hypermineralocorticoidism. Amer. J. Med. **45**, 170 (1968).

CONN, J. W.: Aldosteronism in man. J. Amer. med. Ass. **183**, 775 (1963).

— Plasma renin activity in primary aldosteronism. Importance of differential diagnosis and in research of essential hypertension. J. Amer. med. Ass. **190**, 222 (1964).

— COHEN, E. L., ROONER, D. R.: Suppression of plasma renin activity in primary aldosteronism. Distinguishing primary from secondary aldosteronism in hypertensive disease. J. Amer. med. Ass. **190**, 213 (1964).

— CONN, E. S.: Primary aldosteronism versus hypertensive disease with secondary aldosteronism. Recent Progr. Hormone Res. **17**, 389 (1961).

— KNOPF, R. F., NESBIT, R. M.: Clinical characteristics of primary aldosteronism from an analysis of 145 cases. Amer. J. Surg. **107**, 159 (1964).

KAPLAN, N. M.: Primary aldosteronism with malignant hypertension. New Engl. J. Med. **269**, 1282 (1963).

MILNE, M. D., MUEHRCKE, R. C., AIRD, J.: Primary aldosteronism. Quart. J. Med. **26**, 317 (1957).

PRUNTY, F. T. G.: Aldosteronism — primary and secondary. In: Modern trends in endocrinology, Second Series, ed. GARDINER-HILL, H. London: Butterworth 1961.

RELMAN, A. S.: Diagnosis of primary aldosteronism. Amer. J. Surg. **107**, 159 (1964).

SIEGENTHALER, W.: Klinische Syndrome mit Hyperaldosteronismus unter besonderer Berücksichtigung pathogenetischer Gesichtspunkte. Schweiz. med. Wschr. **93**, 1803 (1963).

— MANN, M., GFELLER, J., GANZONI, A.: Aldosteronforschung und klinische Medizin. Schweiz. med. Wschr. **94**, 685 (1964).

SYMINGTON, TH.: Functional pathology of the human adrenal gland. Edinburgh-London: Livingstone 1969.

WOLFF, H. P.: Aldosteron und Aldosteronismus. Klin. Wschr. **42**, 711 (1964).

Primary Aldosteronism (Conn's Syndrome and Differential Diagnosis)

BIGLIERI, E. G., FORSHAM, P. H.: Studies on the expanded extracellular fluid and the responses to various stimuli in primary aldosteronism. Amer. J. Med. **30**, 564 (1961).

— HANE, S., STATON, P. E., JR., FORSHAM, P. H.: *In vivo* and *in vitro* studies of adrenal secretions in Cushing' syndrome and primary aldosteronism. J. clin. Invest. **42**, 516 (1963).

— SLATON, P. E., JR., FORSHAM, P. H.: Useful parameters in the diagnosis of primary aldosteronism. J. Amer. med. Ass. **178**, 19 (1961).

BROWN, J. J., DAVIES, D. L., LEVER, A. F., ROBERTSON, J. I. S.: Renin and angiotensin. A survey of some aspects. Postgrad. med. J. **42**, 153 (1966).

CONN, J. W.: Primary aldosteronism. II. A new clinical syndrome. J. Lab. clin. Med. **45**, 6 (1955).

— COHEN, E. L., ROVNER, D. R., NESBIT, R. M.: Normokalaemic primary aldosteronism. A detectable cause of curable "essential" hypertension. J. Amer. med. Ass. **193**, 200 (1965).

— LOUIS, L. H.: Primary aldosteronism, a new clinical entity. Ann. intern. Med. **44**, 1 (1956).

— MORITA, R., COHEN, E. L., BEIERWALTES, W. H., MCDONALD, J., HERWIG, K. R.: Primary aldosteronism. Photoscanning of tumors after administration of ^{131}I-19-iodocholesterol. Arch. int. Med. **129**, 417 (1972).

— ROVNER, D. R., COHEN, E. L.: Licorice-induced pseudoaldosteronism. J. Amer. med. Ass. **205**, 492 (1968).

— — — BOOKSTEIN, J. J., CERNY, J. C., LUCAS, C. P.: Preoperative diagnosis of primary aldosteronism. Arch. intern. Med. **123**, 113 (1969).

— — — NESBIT, R. M.: Normokalaemic primary aldosteronism; its masquerade as "essential" hypertension. J. Amer. med. Ass. **195**, 21 (1966).

ESPINER, E. A., TUCCI, J. R., JAGGER, P. J., LAULER, D. P.: Effect of saline infusions on aldosterone and electrolyte excretion in normal subjects and patients with primary aldosteronism. New Engl. J. Med. **277**, 1 (1967).

FISHMAN, L. M., KÜCHEL, O., LIDDLE, G. W., MICHELAKIS, A. M., GORDON, R. D., CHICK, W. T.: Incidence of primary aldosteronism in uncomplicated "essential" hypertension. J. Amer. med. Ass. **205**, 497 (1968).

FOYE, L. V., JR., FEICHTMEIR, TH. V.: Adrenal cortical carcinoma producing solely mineralocorticoid effect. Amer. J. Med. **19**, 966 (1955).

FRITZ, K. W., BÖHM, P.: Primärer Aldosteronismus. Dtsch. med. Wschr. **31**, 1505 (1963).

GARDNER, J. D., LAPEY, A., SIMOPOULOS, A. P., BRAVO, E. L.: Abnormal membrane sodium transport in Liddle's syndrome. J. clin. Invest. **50**, 2253 (1971).

GARST, J. B., SHUMWAY, N. P., SCHWARTZ, H., FARRELL, G. L.: Aldosterone excretion in essential hypertension. J. clin. Endocr. **20**, 1351 (1960).

GEORGE, J. M., WRIGHT, L., BELL, N. H., BARTTER, F. C.: The syndrome of primary aldosteronism. Amer. J. Med. **48**, 343 (1970).

GRANDCHAMP, A., SCHERRER, J. R., VEYRAT, R., MÜLLER, A. F.: I. Measurement of sweat sodium and potassium excretion for evaluation of mineralocorticoid activity in normal subjects. Helv. med. Acta **34**, 367 (1969).

HÖKFELT, B.: Der primäre Aldosteronismus. Verh. dtsch. Ges. inn. Med. 68. Kongr., S. 616, 1962.

JENNY, M., MÜLLER, A. F., FABRE, J., MACH, R. S.: Hypokaliémie et alcalose par ingestion abusive d'extrait de réglisse (Liquorice) et d'eau bicarbonatée. Schweiz. med. Wschr. **91**, 869 (1961).

KAPLAN, N. M.: The steroid content of adrenal adenomas and measurements of aldosterone production in patients with essential hypertension and primary aldosteronism. J. clin. Invest. **46**, 728 (1967).

— SILAK, J. G.: Angiotensin-infusion test: New approach to differential diagnosis of renovascular hypertension. New Engl. J. Med. **271**, 536 (1964).

KOCZOREK, K. R.: Primärer Aldosteronismus (Conn-Syndrom). Internist (Berl.) **5**, 32 (1964).

LARAGH, J. H., KELLY, W. G.: Abnormal aldosterone secretion in disease and its implications. In: Adv. Met. disorders, ed. by R. LEVINE and R. LUFT, vol. I, p. 236. New York: Academic Press 1964.

— SEALEY, J. E., SOMMERS, S. C.: Patterns of adrenal secretion and urinary excretion of aldosterone and plasma renin activity in normal and hypertensive subjects. Circulat. Res., Suppl. I, **18/19**, 158 (1966).

LAULER, D. P.: Preoperative diagnosis of primary aldosteronism. Amer. J. Med. **41**, 855 (1966).

LENNON, E. J., RUETZ, PH. P., ENGSTROM, W. W.: Reversal of diurnal rhythm in excretion of water and salt in primary hyperaldosteronism. Amer. J. med. **30**, 475 (1961).

LIDDLE, G. W., BLEDSOE, T., COPPAGE, W.: A familial renal disorder simulating primary aldosteronism but with negligible Aldosterone secretion. Symposium on Aldosterone. Oxford: Blackwell Scientific Publ. Ltd. 1964.
LUETSCHER, J. A.: Primary aldosteronism: observations in six cases and review of diagnostic procedures. Medicine (Baltimore) **43**, 437 (1964).
MACH, R. S.: Le rôle des surrénales dans l'hypertension artérielle. Schweiz. med. Wschr. **92**, 1027 (1962).
MADER, I. W., ISERI, L. T.: Spontaneous hypopotassemia, hypomagnesemia, alkalosis and tetania due to hypersecretion of corticosterone-like mineralocorticoid. Amer. J. Med. **19**, 976 (1955).
MELBY, J. C., SPARK, R. F., DALE, S. L., EGDAHL, R. H., KAHN, P. C.: Diagnosis and localization of aldosterone-producing adenomas by adrenal vein catheterization. New Engl. J. Med. **277**, 1050 (1967).
MÜLLER, A. F., MANNING, E. L., HODLER, J.: Hypokaliämie, Aldosteron-Ausscheidung und primärer Hyperaldosteronismus. Schweiz. med. Wschr. **93**, 1265 (1963).
NEW, M. I., PETERSON, R. E.: Disorders of aldosterone secretion in childhood. Pediat. Clin. N. Amer. **13**, 43 (1966).
ROSS, E. J., CRABBÉ, J., RENOLD, A. E., EMERSON, K., JR., THORN, G. W.: A case of massive edema in association with an aldosterone-secreting adrenocortical adenoma. Amer. J. Med. **25**, 278 (1958).
ROSSIER, P. H., STAEHELIN, D., BÜHLMANN, A., LABHART, A.: Alkalose und Hypokaliämie bei Anorexia mentalis (Hungeralkalose). Schweiz. med. Wschr. **85**, 465 (1955).
SCHEITLIN, W.: Die renal-vaskuläre Hypertonie. Ergebn. inn. Med. Kinderheilk. **26**, 45 (1967).
SHIELDS, R., MILES, J. B., GILBERTSON, C.: Absorption and secretion of water and electrolytes by the intact colon in a patient with primary aldosteronism. Brit. med. J. **1968 I**, 93.
SIEGENTHALER, W., BAUMANN, K., KIEPENHEUER, K., SCHÖNBECK, M., RHOMBERG, F., GYSLING, E., WEIDMANN, P., WERNING, C.: Die Bedeutung des primären Aldosteronismus als Ursache der „essentiellen" Hypertonie. Schweiz. med. Wschr. **99**, 825 (1969).
— MÖHRING, J., WEIDMANN, P.: Zur Diagnostik des Conn-Syndroms. Dtsch. med. Wschr. **92**, 1569 (1967).
SMITHWICK, R. H., HARRISON, J. H., UNGER, L., WHITELAW, G. P.: Surgical treatment of aldosteronism. Amer. J. Surg. **167**, 178 (1964).
TONUTTI, E., BAYER, J. M.: Beitrag zur Kenntnis der Struktur der NNR-Adenome und der NNR beim primären Aldosteronismus. Endokrinologie **45**, 276 (1963).
VEYRAT, R., FABRE, J., MÜLLER, A. F.: Inhibition sélective de la sécrétion de l'aldostérone par un héparinoïde semi-synthétique (Ro I 8307). Helv. med. Acta **29**, 543 (1962).

Secondary Aldosteronism with Edema

BÖHM, P., FRANKEN, F. H., IRMSCHER, K.: Beitrag zum Problem des idiopathischen Ödems. Klin. Wschr. **41**, 1155 (1963).
HICKIE, J. B., LAZARUS, L.: Aldosterone metabolism in cardiac failure. Aust. Ann. Med. **15**, 289 (1966).
MACH, R. S., FANCONI, A., MÜLLER, A. F., BERNHEIM, PH., NEHER, R.: Hyperaldosteronisme avec hyperplasie adénomateuse des surrénales chez un hypertendu avec oedèmes réfractaires. Schweiz. med. Wschr. **89**, 98 (1959).
MÜLLER, A. F.: Aldostérone et oedème. Schweiz. med. Wschr. **89**, 1093 (1959).
STREETEN, D. H. P., LOUIS, L. H., CONN, J. W.: Secondary aldosteronism in "idiopathic edema". Trans. Ass. Amer. Phycns. **23**, 227 (1960).
THORN, G. W.: Cyclic edema. Amer. J. Med. **23**, 507 (1957).
— Aproach to the patient with "idiopathic edema" or "periodic swelling". J. Amer. med. Ass. **206**, 333 (1968).
VEYRAT, R., MÜLLER, A. F., MACH, R. S.: Les hyperaldostéronismes secondaires. Schweiz. med. Wschr. **98**, 65 (1968).
— ROBERT, M., MACH, R. S.: Étude de la rénine dans les oedèmes idiopathiques avec hyperaldostéronique secondaire. Schweiz. med. Wschr. **98**, 1499 (1968).

Secondary Aldosteronism due to Angiotensin

ALEXANDER, M., PRAETORIUS, F.: Zur Abgrenzung des „Bartter-Syndroms". Dtsch. med. Wschr. **92**, 1022 (1967).
BARTTER, F. C., PRONOVE, P., GILL, J. R., JR., MACCARDLE, R. C.: Hyperplasia of the iuxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis. Amer. J. Med. **33**, 811 (1962).
BEILIN, L. J., SCHIFFMANN, N., CRANE, M., NELSON, D. H.: Hypokalemic alkalosis and hyperplasia of the juxtaglomerular apparatus without hypertension or oedema. Brit. med. J. **1967 II**, 327.
GOODMAN, A. D., VAGNUCCI, A. H., HARTROFT, P. M.: Pathogenesis of Bartter's syndrome. New Engl. J. Med. **281**, 1435 (1969).
LAIDLAW, J. C., YENDT, E. R., GORNALL, A. G.: Hypertension caused by renal artery occlusion simulating primary aldosteronism. Metabolism **9**, 612 (1960).
LARAGH, J. H., SEALEY, J. E., SOMMERS, S. C.: Patterns of adrenal secretion and urinary excretion of aldosterone and plasma renin activity in normal and hypertensive subjects. Circulat. Res., Suppl. **1**, vols. 18, 19, p. 158 (1966).
ROYER, P., HABIB, R., MATHIEU, H.: Nephrologie im Kindesalter. Stuttgart: Thieme 1967.
SCHWAB, M. R., SCHRÖDER, C., SCHATTENFROH, C., SCHÜTZ, R. M.: Sekundärer Aldosteronismus unter dem Bild des Conn-Syndroms bei einseitiger Nierenarterienstenose. Dtsch. med. Wschr. **88**, 814 (1963).

ACTH-Dependent Secondary Aldosteronism

NEW, M. I., PETERSON, R. E.: A new form of congenital adrenal hyperplasia. J. clin. Endocr. **27**, 300 (1967).
SUTHERLAND, D. J. A., RUSE, J. L., LAIDLAW, J. C.: Hypertension, increased aldosterone secretion and low plasma renin activity relieved by dexamethasone. Canad. med. Ass. J. **95**, 1109 (1966).

Secondary Aldosteronism of Unknown Etiology

BAER, L., SOMMERS, S. C., KRAKOFF, L. R., NEWTON, M. A., LARAGH, J. H.: Pseudo-primary aldosteronism; an entity distinct from true primary aldosteronism. Circulat. Res., Suppl. 1, **27**, 1—203 (1970).
DAVIS, W. W., NEWSOME, H. H., JR., WRIGHT, L. D., JR., HAMMOND, W. G., EASTON, J., BARTTER, F. C.: Bilateral adrenal hyperplasia as a cause of primary aldosteronism with hypertension, hypokalemia and suppressed renin activity. Amer. J. Med. **42**, 642 (1967).
SALTI, I. S., RUSE, J. L., STIEFEL, M., DELARUE, N. C., LAIDLAW, J. C.: Non-tumorous "primary" aldosteronism: II. Type not relieved by glucocorticoid. Canad. med. Ass. J. **101**, 11 (1969).

17-Hydroxylase Deficiency

BIGLIERI, E. G., HERRON, M. A., BRUST, N.: 17-Hydroxylation deficiency in man. J. clin. Invest. **45**, 1946 (1966).
GOLDSMITH, O., SOLOMON, D. H., HORTON, R.: Hypogonadism and mineralocorticoid excess. The 17-hydroxylase deficiency syndrome. New Engl. J. Med. **277**, 673 (1967).
NEW, M. I.: Male pseudohermaphroditism due to 17α-hydroxylase deficiency. J. clin. Invest. **49**, 1930 (1970).

Hyperdeoxycorticosteronism

BIGLIERI, E. G.: Hypokalemic alkalosis and edema with increased desoxycorticosterone excretion. J. clin. Endocr. **25**, 884 (1965).

18-OH-DOC Excess

MELBY, J. C., DALE, S. L., GREKIN, R. L., GAUNT, R., WILSON, T. E.: 18-Hydroxy-11-deoxycorticosterone (18-OH-DOC) secretion in experimental and human hypertension. Recent Progr. Hormone Res. **28**, 287 (1972).

Cushing's Syndrome

Reviews

BINDER, E., HALL, P. (eds.): Cushing's syndrome. Diagnosis and treatment. London: Heinemann 1972.
CUSHING, H.: The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). Bull. Johns Hopk. Hosp. **50**, 137 (1932).
HERRERA, M. G., CAHILL, G. F., JR., THORN, G. W.: Cushing's syndrome. Diagnosis and treatment. Amer. J. Surg. **107**, 144 (1964).
KARL, H. J.: Das Cushing-Syndrom. Internist (Berl.) **5**, 1 (1964).
LABHART, A.: Die Nebennierenrinden-Überfunktionssyndrome. Ärztl. Fortbild. **11**, 590 (1961).
— Das Cushing-Syndrom. Öst. Ärztezg. **2**, 125 (1966).
PETERSON, R. E.: Das Cushingsche Syndrom. Praxis **52**, 134 (1963).
PLOTZ, CH. M., KNOWLTON, A. I., RAGAN, CH.: The natural history of Cushing's syndrome. Amer. J. Med. **13**, 597 (1952).
SOULAIRAC, A.: Le syndrome de Cushing. Rapports de la IX^e réunion des endocrinologistes de langue française. Paris: Doin 1967.

Etiology, Pathogenesis and Pathologic Anatomy

ALBRIGHT, F.: Cushing's syndrome. Harvey Lect., Ser. **38**, 123 (1942/43).
BIGLIERI, E. G., HANE, S., SLATON, P. E., JR., FORSHAM, P. H.: *In vivo* and *in vitro* studies of adrenal secretions in Cushing's syndrome and primary aldosteronism. J. clin. Invest. **42**, 516 (1963).
FABRYKANT, M., JACKSON, R. S., ASKE, B. I.: Cushing's syndrome: failure to demonstrate diminished peripheral glucose uptake and insulin resistance. Metabolism **6**, 116 (1957).
FROST, H. M.: Bone remodelling dynamics. Springfield: Ch. C. Thomas 1963.
GUINET, P., MORNEX, R., REVOL, A., BERTRAND, J., VEYRAT, A., DORSIT, G.: Considérations sur deux observations de maladie de Cushing avec syndrome d'alcalose hypokaliémique. Rev. lyon. Méd. **16**, 5 (1967).
HENNEMANN, D. H., BUNKER, J. P.: The pattern of intermediary carbohydrate metabolism in Cushing's syndrome. Amer. J. Med. **23**, 34 (1957).
HOWARD, J. E., MIGEON, C. J.: Cushing's syndrome produced by normal replacement doses of cortisone in patient with defective mechanism for steroid degradation. Amer. J. med. Sci. **235**, 387 (1958).
KRACHT, J., TAMM, J.: Invasiv gewachsenes basophiles Adenom des Hypophysenvorderlappens bei Cushing-Syndrom. Acta endocr. (Kbh.) **32**, 330 (1963).
LIDDLE, G. W., ISLAND, D., MEADOR, C. K.: Normal and abnormal regulation of corticotropin secretion in man. Recent Progr. Hormone Res. **18**, 125 (1962).
— WILLIAMS, W. C., JR., WALSER, A.: Die Bedeutung von ACTH für die Pathogenese der Cushingschen Erkrankung. Schweiz. med. Wschr. **90**, 1325 (1960).
MARKS, V.: Cushing's syndrome occuring with pituitary chromophobe tumors. Acta endocr. (Kbh.) **32**, 527 (1959).
NELSON, D. H., SPRUNT, J. G., MIMS, R. B.: Plasma ACTH determinations in 58 patients before or after adrenalectomy for Cushing's syndrome. J. clin. Endocr. **26**, 722 (1966).
NEY, R. L., SHIMIZU, N., NICHOLSON, W. E., ISLAND, D. P., LIDDLE, G. W.: Correlation of plasma ACTH concentration with adrenocortical response in normal human subjects, surgical patients and patients with Cushing's disease. J. clin. Invest. **42**, 1669 (1963).
PRUNTY, F. T. G.: Chemistry and treatment of adrenocortical diseases. Springfield: Thomas 1964.
RETIENE, K., ESPINOZA, A., MARX, K. H., PFEIFFER, E. F.: Über das Verhalten von ACTH und Cortisol im Blut von Normalen und Kranken mit primärer und sekundärer Störung der Nebennierenrindenfunktion. I. Nachweis der vermehrten ACTH-Sekretion beim Morbus Cushing. Klin. Wschr. **43**, 205 (1965).
SYMINGTON, TH.: Functional pathology of the human adrenal gland. Edinburgh-London: Livingstone 1969.
THOMPSON, K. W., EISENHARDT: Further consideration of the Cushing's syndrome. J. clin. Endocr. **3**, 445 (1943).

Clinical Features, Symptomatology and Diagnostic Procedures

BAYER, J. M., NEUNER, H. P.: Cushing-Syndrom und erhöhter Augeninnendruck. Dtsch. med. Wschr. **92**, 1791 (1967).
BEIERWALTES, W. H., LIEBERMAN, L. M., ANSARI, A. N., NISHIYAMA, H.: Visualization of human adrenal glands *in vivo* by scintillation scanning. J. Amer. med. Ass. **216**, 275 (1971).
BLEULER, M.: Endokrinologische Psychiatrie. In: Psychiatrie der Gegenwart, Bd. I/13. Berlin-Göttingen-Heidelberg: Springer 1964.
CAMANNI, F., LOSANA, O., MASSASA, F., MOLINATTI, G. M.: Increased renal phosphate excretion in Cushing's syndrome. Acta endocr. (Kbh.) **56**, 85 (1965).
COST, W. S.: Quantitative estimation of adrenocortical hormones and their α-ketolic metabolites in urine. II. Pathologic adrenocortical hyperfunction. Acta endocr. (Kbh.) **42**, 39 (1963).
EKMAN, H., HÅKANSSON, B., MCCARTHY, J. D., LEHMANN, J., SJÖGREN, B.: Plasma 17-hydroxycorticosteroids in Cushing's syndrome. J. clin. endocr. **21**, 684 (1961).
FLURY, A., MÜLLER, J., FROESCH, E. R., LABHART, A.: Das Cushing-Syndrom. Kasuistische Zusammenstellung von 43 Cushing-Patienten der Medizinischen Univ.-Klinik Zürich von 1958—1969. Schweiz. med. Wschr. (im Druck).
FURGER, R.: Psychiatrische Untersuchungen beim Cushing-Syndrom. Schweiz. Arch. Neurol. Psychiat. **88**, 9 (1961).
GUINET, P., MORNEX, R., REVOT, A., BERTRAND, J., VEYRAT, A., DORSIT, G.: Considérations sur deux observations de maladie de Cushing avec syndrome d'alcalose hypokaliémique. Rev. lyon. Méd. **16**, 5 (1967).
IANNACONE, A.: The ovaries in Cushing's syndrome. New Engl. J. Med. **261**, 775 (1959).
— GABRILOVE, J. L., BRAHMS, S. A., SOFFER, L. J.: Roentgen diagnosis of adrenal tumor in Cushing's syndrome. Arch. intern. Med. **105**, 257 (1960).
— — — — Osteoporosis in Cushing's syndrome. Ann. intern. Med. **52**, 570 (1960).
LABHART, A., FROESCH, E. R., ZIEGLER, W.: Zur Diagnose und Therapie des Cushing-Syndroms. Schweiz. med. Wschr. **89**, 44 (1959).

LIDDLE, G. W.: Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. J. clin. Endocr. **12**, 1539 (1960).
MOLINATTI, G. M., CAMANNI, F., OLIVETTI, M.: A study on the metabolism of calcium in the hyperadrenocortical syndrome. Acta endocr. (Kbh.) **34**, 323 (1960).
MURRAY, R. O.: Radiologic bone changes in Cushing's syndrome and steroid therapy. Brit. J. Radiol. **33**, 1 (1960).
NELSON, D. H., SPRUNT, J. G., MIMS, R. B.: Plasma ACTH determinations in 58 patients before or after adrenalectomy for Cushing's syndrome. J. clin. Endocr. **26**, 722 (1966).
NICHOLS, TH., NUGENT, CH., A., TYLER, F. H.: Steroid laboratory tests in the diagnosis of Cushing's syndrome. Amer. J. Med. **45**, 116 (1968).
NUGENT, CH. A.: Probability theory in the diagnosis of Cushing's syndrome. J. clin. Endocr. **24**, 621 (1964).
PRUNTY, F. T. G.: Chemistry and treatment of adrenocortical diseases. Springfield: Thomas 1964.
ROSS, E. J., MARSHALL-JONES, P., FRIEDMAN, M.: Cushing's syndrome: Diagnostic criteria. Quart. J. Med., N.S. **35**, 149 (1966).
ROVIT, R. L., DUANE, T. D.: Cushing's syndrome and pituitary tumors. Pathophysiology and ocular manifestations of ACTH-secreting pituitary adenomas. Amer. J. Med. **46**, 416 (1969).
SALASSA, R. M., KEARNS, TH. P., KERNOHAN, J. W., SPRAGUE, R. G., MCCARTHY, C. S.: Pituitary tumors in patients with Cushing's syndrome. J. clin. Endocr. **19**, 1523 (1959).
SCHWARZ, F., KINDEREN, P. J. DER, HOUTSRA-LANZ, M.: Exophthalmos producing activity in the serum and in the pituitary of patients with Cushing's syndrome and acromegaly. J. clin. Endocr. **22**, 718 (1962).
SOFFER, L. J., IANNACONE, A., GABRILOVE, J. L.: Cushing's syndrome. A study of fifty patients. Amer. J. Med. **30**, 129 (1961).
STEINBACH, H. L., NOETZLI, M., OZONOFF, M. B.: Small pituitary fossa in Cushing's syndrome due to adrenal neoplasm. New Engl. J. Med. **24**, 1286 (1963).
WEST, CH. D., DAMAST, B., PEARSON, O. H.: Urinary estrogen excretion in Cushing's syndrome. J. clin. Endocr. **18**, 15 (1958).

X-Ray Diagnosis

BUETTI-BÄUML, C.: Über eine neue Technik in der Röntgendiagnostik: Das Pneumoretroperitoneum. Radiol. clin. (Basel) **21**, 225 (1952).
COCCHI, U.: Retropneumoperitoneum und Pneumomediastinum. Fortschr. Röntgenstr., Erg.-Bd. **1956**.
Editorial: Adrenal venography. New Engl. J. Med. **26**, 1453 (1968).
REUTER, S. R.: Demonstration of adrenal metastases by adrenal venography. New Engl. J. Med. **26**, 1423 (1968).
STEINBACH, H. L., SMITH, D. R.: Extraperitoneal pneumography in diagnosis of retro-peritoneal tumors. Arch. Surg. **70**, 161 (1955).
VOEGELI, E.: Die Nebennierenphlebographie. Schweiz. med. Wschr. **100**, 1517 (1970).

Adrenocortical Carcinoma

LIPSETT, M. B., HERTZ, R., ROSS, G. T.: Clinical and pathophysiologic aspects of adrenocortical carcinoma. Amer. J. Med. **35**, 374 (1963).
MACFARLANE, D. A.: Cancer of the adrenal cortex. Ann. roy. Coll. Surg. Engl. **23**, 155 (1958).

Cushing's Syndrome due to Ectopic Corticotropin Production in Tumors (Paraneoplastic Cushing's Syndrome) and by Mixed Cortical Medullary Tumors

ALLOT, E. N., SHELTON, M. O.: Increased adrenocortical activity associated with malignant disease. Lancet **1964II**, 278.
BAGSHAWE, K. D.: Hypokaliaemia, carcinoma and Cushing syndrome. Lancet **1960II**, 284.
BRICKNER, PH. W., LYONS, M., LANDAU, S. J.: Cushing's syndrome associated with non-endocrine neoplasms. Amer. J. Med. **31**, 632 (1961).
BROOKS, R. V., DUPRÉ, J., GOGATE, A. N., MILLS, I. H., PRUNTY, F. T. G.: Appraisal of adrenocortical hyperfunction: patients with Cushing's syndrome or "non-endocrine" tumors. J. clin. Endocr. **23**, 725 (1963).
COHEN, R. D., ROSS, I. P., DAYAN, A. D.: Metabolic studies in a case of adrenocortical hyperfunction associated with carcinoma of the lung. J. clin. Endocr. **24**, 401 (1964).
COST, W. S.: A mineralocorticoid excess syndrome presumably due to excessive secretion of corticosterone. Lancet **1963I**, 362.
CRANE, M. G., HARRIS, J. J.: Desoxycorticosterone secretion rates in hyperadrenocorticism. J. clin. Endocr. **26**, 1135 (1966).
FRIEDMAN, M., MARSHALL-JONES, P., ROSS, E. J.: Cushing's syndrome: adrenocortical hyperactivity secondary to neoplasms arising outside the pituitary-adrenal system. Quart. J. Med. **35**, 193 (1966).
GREENBERG, E., DIVERTIE, M. B., WOOLNER, L. B.: A review of unusual systemic manifestations associated with carcinoma. Amer. J. Med. **36**, 106 (1964).
HAAS, H. G.: Endokrine Überfunktion bei malignen Tumoren. Praxis **53**, 610 (1964).
HALLWRIGHT, G. P., NORTH, K. A. K., REID, J. D.: Pigmentation and Cushing's syndrome due to malignant tumor of the pancreas. J. clin. Endocr. **24**, 496 (1964).
HARRISON, M. T., MONTGOMERY, D. A. D., RAWSEY, A. S., ROBERTSON, J. H., WELBOURN, R. B.: Cushing's syndrome with carcinoma of bronchus, and with feature suggesting carcinoid tumor. Lancet **1957I**, 23.
JARETT, L., LACY, P. E., KIPNIS, D. M.: Characterization by immuno-fluorescence of an ACTH-like substance in non-pituitary tumors from patients with hyperadrenocorticism. J. clin. Endocr. **24**, 543 (1964).
KOVACH, R. D., KYLE, L. H.: Cushing's syndrome and bronchogenic carcinoma. Amer. J. Med. **24**, 981 (1958).
KRACHT, J., HANTSCHMANN, N.: Tumorsyntropien des Cushing-Syndroms. Acta endocr. (Kbh.) **38**, 490 (1961).
— PFOTENHAUER, R.: Die extrahypophysären corticotropen Geschwülste. 12. Symp. der deutschen Ges. für Endokrinologie. p. 274. Berlin-Heidelberg-New York: Springer 1967.
LABHART, A.: Endokrine Überfunktionssyndrome der Gewebehormone. 8. Symposion Dtsch. Ges. Endokrinologie, in München 1961, p. 77. Berlin-Göttingen-Heidelberg: Springer 1962.
LIDDLE, G. W., ISLAND, D. P., NEY, R. L., NICHOLSON, W. E., SHIMIZU, N.: Nonpituitary neoplasms and Cushing's syndrome. Arch. intern. Med. **111**, 471 (1963).
MACH, R. S., RENTCHNIK, P., MÜLLER, A. F., LAGIER, J., PLATTNER, H. C.: Syndrome humoral d'hypercorticisme avec alcalose et hyperkaliémie par métastases surrénaliennes de carcinomes bronchiques. Presse méd. **66**, 437 (1958).
MATHISON, D. A., WATERHOUSE, C. A.: Cushing's syndrome with hypertensive crisis and mixed adrenal cortical adenoma-pheochromocytoma (corticomedullary adenoma). Amer. J. Med. **47**, 635 (1969).
MELONI, C. R., TUCCI, J., CANARY, J. J., KYLE, L. H.: Cushing's syndrome due to bilateral adrenocortical hyperplasia

caused by a benign adrenal medullary tumor. J. clin. Endocr. **26**, 1192 (1962).
NICHOLS, J.: ACTH like excretion from carcinoma of the ovary. J. Amer. med. Ass. **182**, 713 (1962).
O'RIORDAN, J. L. H., BLANSHARD, G. P., MOXHAM, A., NABARRO, J. D. N.: Corticotropin-secreting carcinomas. Quart. J. Med. **35**, 137 (1966).
PRUNTY, F. T. G., BROOKS, R. V., DUPRÉ, J., GIMLETTE, T. M. D., HUTCHINSON, J. S. M., MCSWINEY, R. R., MILLS, I. H.: Adrenocortical hyperfunction and potassium metabolism in patients with "non-endocrine" tumors and Cushing's syndrome. J. clin. Endocr. **23**, 737 (1963).
RIGGS, B. L., SPRAGUE, R. G.: Association of Cushing's syndrome and neoplastic disease. Arch. intern. Med. **108**, 841 (1961).
STROTT, CH. A., NUGENT, CH. A., TYLER, F. H.: Cushing's syndrome caused by bronchial adenomas. Amer. J. Med. **44**, 97 (1968).

Transient and Periodic Cushing's Syndrome

ABER, C. P., CHEETHAM, H. D.: Cyclical Cushing's syndrome. Brit. med. J. **1961 I**, 336.
BROOKS, R. V., JEFFWATE, S. L., LONDON, D. R., PRUNTY, F. T. G., SMITHS, P. M.: Intermittent Cushing's syndrome with anomalous response to dexamethasone. J. Endocr. **36**, 53 (1966).
BROWN, R. D., VAN LOON, G. R., ORTH, D. N., LIDDLE, G. W.: Cushing's disease with periodic hormonogenesis: One explanation for paradoxical response to dexamethasone.
HAYSLETT, J. P., COHN, G. L.: Spontaneous remission of Cushing's disease. Report of a case. New Engl. J. Med. **276**, 968 (1967).
HONDEK, H., LESZYNSKY, H. E.: Transient Cushing syndrome. Brit. med. J. **1956 I**, 197.
ÖVLISEN, B., ANDERSEN, H. J.: Spontaneous remission in case of Cushing's syndrome presumably due to adrenal tumor. J. clin. Endocr. **26**, 294 (1966).
PASQUALINI, GUREVICH, N.: Spontaneous remission in a case of Cushing's syndrome. J. clin. Endocr. **16**, 406 (1956).
SCHWAB, R., DENNINGER, K.: Das transitorische Cushing-Syndrom. Ergebn. inn. Med. Kinderheilk. **12**, 563 (1959).
WOLFF, S. M., ADLER, R. C., BUSKIRK, E. R., THOMPSON, R. H.: A syndrome of periodic hypothalamic discharge. Amer. J. Med. **36**, 956 (1964).

Recurrent Cushing's Syndrome after Total Bilateral Adrenalectomy

CHAFFEE, W. R., MOSES, A. M., LLOYD, C. W., ROGERS, L. S.: Cushing's syndrome with acessory adrenocortical tissue. J. Amer. med. Ass. **186**, 799 (1963).
ENGEL, F. L., MCPHERSON, H. T., FETTER, B. F., BAGGETT, B., ENGEL, L. L., CARTER, P., FIELDING, L. L., SAVARD, K., DORFMAN, R. I.: Clinical, morphological and biochemical studies on a malignant testicular tumor. J. clin. Endocr. **24**, 528 (1964).
KOZAK, G. P., PAUK, G. L., VAGNUCCI, A. I., LAULER, D. P., THORN, G. W.: Adrenal secretion after bilateral adrenalectomy for Cushing's syndrome. Ann. intern. Med. **64**, 778 (1966).
NEY, R. L., HAMMOND, W., WRIGHT, L., DAVIS, W. W., ACHER, J., BARTTER, F. C.: Studies in a patient with an ectopic adrenocortical tumor. J. clin. Endocr. **26**, 299 (1966).
STAUB, J.-J.: Rezidiv eines Cushing-Syndromes nach totaler Adrenalektomie und Hypophysenausschaltung. Ektopische Hormonbildung durch Pankreasadenom. Inaug.-Diss. Basel 1967.

Primary Nodulary Dysplasia of the Adrenal Cortex

MEADOR, C. K., BOWDOIN, B., OWEN, W. C., JR., FARMER, T. A., JR.: Primary adrenocortical nodular dysplasia: a rare cause of Cushing's syndrome. J. clin. Endocr. **27**, 1255 (1967).

Cushing's Syndrome and Pregnancy

BERGMAN, P., EKMAN, H., HÅKANSSON, B., SJÖGREN, B.: Adrenalectomy during pregnancy with the appearance of preeclampsia at term in a case of Cushing's syndrome. Acta endocr. (Kbh.) **35**, 293 (1960).
EISENSTEIN, A. B., KARSCH, R., GALL, I.: Occurence of pregnancy in Cushing's syndrome. J. clin. Endocr. **23**, 971 (1963).
KREINES, K., PERRIN, E., SALZER, R.: Pregnancy in Cushing's syndrome. J. clin. Endocr. **24**, 75 (1964).
MOLINATTI, G. M., OLIVETTI, M.: Surrenectomia durante la gravidanza in un caso di sindrome di Cushing. Min. Med. **147**, 1191 (1963).
PARRA, A., CRUZ-KROHN, J.: Intercurrent Cushing's syndrome and pregnancy. Amer. J. Med. **40**, 961 (1966).

Differential Diagnosis against Simple Obesity

DUNKELMAN, S. S., FAISHURST, B., PLAYER, J., WATERHOUSE, CH.: Cortisol metabolism in obesity. J. clin. Endocr. **24**, 832 (1964).
Editorial: Steroid paintbrush. New Engl. J. Med. **266**, 1065 (1962).
GOGATE, A. N., PRUNTY, F. T. G.: Adrenal cortical function in "obesity with pink striae" in the young adult. J. clin. Endocr. **23**, 747 (1963).
JACOBSON, G., SELTZER, C. C., BONDY, PH. K., MAYER, J.: Importance of body characteristics in the excretion of 17-ketosteroids and 17-ketogenic steroids in obesity. New Engl. J. Med. **271**, 651 (1964).
MIGEON, C. J., GREEN, O. C., ECKERT, J. P.: Study of adrenocortical function in obesity. Metabolism **12**, 718 (1963).
PREZIO, J. A., CARREON, G., CLERKIN, E., MELONI, C. R., KYLE, L. H., CANARY, J. J.: Influence of body composition on adrenal function in obesity. J. clin. Endocr. **24**, 481 (1964).
SCHTEINGART, D. E., GREGERMAN, R. I., CONN, J. W.: A comparison of the characteristics of increased adrenocortical function in obesity and in Cushing's syndrome. Metabolism **12**, 484 (1963).
SIMKIN, B., ARCE, R.: Steroid excretion in obese patients with colored abdominal striae. New Engl. J. Med. **266**, 1031 (1962).
SUMMERS, V. K., SHEEHAN, H. L., HIPKIN, L. J., DAVIS, J. C.: Differential diagnosis of Cushing's syndrome and obesity associated with striae. Lancet **1964 II**, 1079.

Treatment of Cushing's Syndrome

MELBY, J. C.: Therapeutic possibilities in Cushing's syndrome. New Engl. J. Med. **285**, 288 (1971).
ORTH, D. N., LIDDLE, G. W.: Results of treatment in 108 patients with Cushing's syndrome. New Engl. J. Med. **285**, 243 (1971).

Hormone Therapy

NORMAN, N., VOGT, J. H.: Ethinyloestradiol in the preoperative treatment of a severe case of Cushing's syndrome due to adrenocortical hyperplasia. Acta endocr. (Kbh.) **42**, 168 (1963).

RAUSCH-STROOMANN, J.-G., PETRY, R., TRENKNER, G.: Die Behandlung des Cushingsyndromes auf Grund von Nebennierenrinden-Hyperplasie mit 6-Dehydro-16-methylenhydrocortison. Dtsch. med. Wschr. **93**, 2324 (1968).

Adrenostatic Drugs, o, p'-DDD, Heparinoids

BERGENSTAL, D.M., HERTZ, R., LIPSETT, M.B., MOY, R.H.: Chemotherapy of adrenocortical cancer with o,p'DDD. Ann. intern. Med. **53**, 672 (1960).
FISHER, D.A., PANOS, T.C., MELBY, J.C.: Therapy of adrenocortical cancer with o,p'-DDD in two children. J. clin. Endocr. **23**, 218 (1963).
GALLAGHER, T.F., FUKUSHIMA, D.K., HELLMANN, L.: Effect of o,p'-DDD on steroid hormone metabolites in adrenocortical carcinoma. Metabolism **11**, 1155 (1962).
GLEDSOE, T., ISLAND, D.P., NEY, R.L., LIDDLE, G.W.: An effect of o,p'-DDD on the extra-adrenal metabolism of cortisol in man. J. clin. Endocr. **24**, 1303 (1964).
MOLNAR, G.D., MATTOX, V.R., BAHN, R.C.: Clinical and therapeutic observations in adrenal cancer: report on seven patients treated with o,p'-DDD. Cancer (Philad.) **16**, 259 (1963).
RAUSCH-STROOMANN, J.G.: Treatement of Cushing's syndrome due to adrenal hyperplasia by "chemical hypophysectomy". Acta endocr. (Kbh.), Suppl. **119**, 35 (1967).
VEYRAT, R., FABRE, J., MÜLLER, A.F.: Inhibition sélective de la sécrétion de l'aldostérone par un héparinoide semi-synthétique (Ro I 8707). Helv. med. Acta **29**, 543 (1962).
WEBER, J., DITZEL, J.: Effect of long-term administration of methopyrapone and dexamethasone in patient with Cushing's syndrome. Acta endocr. (Kbh.) **43**, 493 (1963).

Aminoglutethimide

CAMACHO, A.M., CASH, R., BROUGH, A.J., WILROY, R.S.: Inhibition od adrenal steroidogenesis by amino-glutethimide and the mechanism of action. J. Amer. med. Ass. **202**, 20 (1967).
CASH, R., BROUGH, A.J., COHEN, M.N.P., SATOH, P.S.: Aminglutethimide (Elipten-Ciba) as an inhibitor of adrenal steroidogenesis: mechanism of action and therapeutic trial. J. clin. Endocr. **27**, 1239 (1967).
DEXTER, R.N., FISHMAN, L.M., NEY, R.L., LIDDLE, G.W.: Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action. J. clin. Endocr. **27**, 473 (1967).
FISHMAN, L.M., LIDDLE, G.W., ISLAND, D.P., FLEISCHER, N., KÜCHEL, O.: Effects of amino-glutethimide on adrenal function in man. J. clin. Endocr. **27**, 481 (1967).
HORKY, K., KÜCHEL, O., GREGOROVA, I., JIRANKVA, J., MATYS, Z.: Klinische Erfahrungen mit Aminoglutäthimid (Elipten Ciba), einem neuen Inhibitor der Steroid-Biosynthese in der Nebenniere. Schweiz. med. Wschr. **98**, 1843 (1968).
PHILBERT, M., LAUDAT, M.H., LAUDAT, P., BRICAIRE, H.: Etude clinique et biologique d'un inhibiteur de l'hormonosynthèse corticosurrénale: l'aminoglutéthimide. Ann. Endocr. (Paris) **29**, 189 (1968).
RALLISON, M.L., KUMAGAI, , L.F., TYLER, F.H.: Goitrous hypothyroidism induced by amino-glutethimide, anticonvulsant drug. J. clin. Endocr. **27**, 165 (1967).
SMILO, R.P., EARLL, J.M., FORSHAM, P.H.: Suppression of tumorous adrenal hyperfunction by aminoglutethimide. Metabolism **16**, 374 (1967).

Surgical Treatment

COPE, O., RAKER, J.W.: Cushing's disease. The surgical experience in the care of 46 cases. New Engl. J. Med. **253**, 119 (1955).
DINGMAN, J.F., LIM, N.Y.: Cushing's syndrome due to an ACTH-secreting chromophobe adenoma. Report of a case treated by hypophysectomy. New Engl. J. Med. **267**, 696 (1962).
EGDAHL, R.H.: Surgery of the adrenal gland. New Engl. J. med. **278**, 939 (1968).
ESCHER, F.: Die chirurgische Entfernung der Hypophyse. In: Hypophysektomie, Bd. XII, Fortschritt der HNO-Heilkunde. Basel: S. Karger 1965.
HARDY, J.: Transnasal-transsphenoidal approach to the pituitary gland. In: YASARGIL, M.G., Microsurgery applied to neurosurgery. Stuttgart: Thieme; New York-London: Academic Press 1969.
HAYES, M.A., GODENBERG, I.S.: Operative treatment of adrenal cortical hyperfunctioning diseases. Ann. Surg. **154**, 331 (1961).
KRACHT, J., TAMM, J.: Invasiv gewachsenes basophiles Adenom des Hypophysenvorderlappens bei Cushing-Syndrom. Acta endocr. (Kbh.) **32**, 330 (1963).
KYLE, L.H., MEYER, R.J., CANARY, J.J.: Mechanism of adrenal atrophy in Cushing's syndrome due to adrenal tumor. New Engl. J. Med. **257**, 57 (1957).
MASON, A.St.: Treatment of Cushing's syndrome by adrenalectomy. Proc. roy. Soc. Med. **50**, 766 (1957).
MÜLLER, J., FROESCH, E.R., MEYER, U.A., LABHART, A.: Persistierende Störung der ACTH-Sekretion nach Operation eines Nebennierenrinden-Adenoms bei drei Fällen von Cushing-Syndrom. Schweiz. med. Wschr. **97**, 861 (1967).
MUNDINGER, F., RIECHERT, T., REISERT, P.M.: Hypophysentumoren, Hypophysektomie. Stuttgart: Thieme 1967.
NELSON, D.H., MEAKIN, J.W., THORN, G.W.: ACTH-producing pituitary tumors following adrenalectomy for Cushing's syndrome. Ann. intern. Med. **52**, 560 (1960).
— SPRUNT, J.G., MIMS, R.B.: Plasma ACTH determinations in 58 patients before or after adrenalectomy for Cushing's syndrome. J. clin. Endocr. **26**, 722 (1966).
RAND, R.W., DASKE, A.M., PAGLIA, D.E., CONWAY, L.W., SOLOMON, D.H.: Stereotactic cryohypophysectomy. J. Amer. med. Ass. **189**, 255 (1964).
ROBERTS, M.S., LATTIMER, J.K.: The surgical treatment of Cushing's syndrome. J. Amer. med. Ass. **175**, 93 (1961).
SIEBENMANN, R.E.: Invasiv wachsendes, vorwiegend basophiles Adenom des Hypophysenvorderlappens bei Cushing-Rezidiv nach subtotaler Adrenalektomie. Schweiz. Z. allg. Path. **18**, 1189 (1955).
ZERRAS, N.T.: Technique of radio-frequency hypophysectomy. Confin. neurol. (Basel) **26**, 157 (1965).

Radiotherapy

BERINGER, A., BURIAN, K., ELLEGAST, H., FREY, R.G., FRISCHAUF, H., ZEITLHOFER, J.: Die strahlentherapeutische Ausschaltung der Hypophyse. In: F. ESCHER, Hypophysektomie, Bd. XII, Fortschritt der HNO-Heilkunde. Basel: S. Karger 1965.
LINFOOT, J.A., LAWRENCE, J.H., BORN, J.L., TOBIAS, C.A.: The alpha particle or proton beam in radiosurgery of the pituitary gland for Cushing's disease. New Engl. J. Med. **269**, 597 (1963).
MOLINATTI, G.M., CAMANNI, F., TEDESCHI, M.: Implantation of the pituitary with $Yttrium^{90}$ in a case of Cushing's syndrome. J. clin. Endocr. **19**, 1144 (1959).

The Adrenogenital Syndrome

The Congenital Adrenogenital Syndrome

BARTTER, C., HENKIN, R.I., BRYAN, G.T.: Aldosterone hypersecretion in „non-salt-losing" congenital adrenal hyperplasia. J. clin. Invest. **47**, 1742 (1968).

BERGSTRAND, C.G.: Growth in congenital adrenal hyperplasia. Acta paediat. scand. **55**, 463 (1966).
— BIRKE, G., PLANTIN, L.O.: Corticosteroid excretion pattern in infants and children with adrenogenital syndrome. Acta endocr. (Kbh.) **30**, 500 (1959).
BINOUX, M., GIRARD, F., PHAM-HUU-TRUNG, M.T., CANLORBE, P., MOZZICONACCI, P.: La régulation hypophysosurrénale dans l'hyperplasie congénitale des surrénales. Arch. franç. Pédiat. **24**, 369 (1967).
BIRKE, G., DICZFALUSY, E., PLANTIN, L.O., ROBBE, H., WESTMAN, H.: Familial congenital hyperplasia of the adrenal cortex. Acta endocr. (Kbh.) **29**, 55 (1958).
BLIZZARD, R.M., LIDDLE, G.W., MIGEON, C.J., WILKINS, L.: Aldosterone excretion in virilizing adrenal hyperplasia. J. clin. Invest. **38**, 1442 (1959).
BLUNCK, W., BIERICH, J.R.: Congenital adrenal hyperplasia with 11-hydroxylase deficiency. A case report and contribution to diagnosis. Acta paediat. scand. **57**, 157 (1968).
BONATI, B., MARRAMA, P., DELLA CASA, L.: Urinary oestrogens in congenital adrenal hyperplasia. Research on Steroids. Proc. 3rd Meeting of the Internat. Study Group for Steroid Hormones (C. CASSANO, M. FINKELSTEIN, A. KLOPPER, C. CONTI, ed.), vol. III, p. 277. Amsterdam: North Holland Publ. Comp. 1968.
BONGIOVANNI, A.M.: Unusual steroid pattern in congenital adrenal hyperplasia: deficiency of 3β-hydroxy dehydrogenase. J. clin. Endocr. **21**, 860 (1961).
— Renin activity, aldosterone secretion, and congenital adrenal hyperplasia. Pediatrics **41**, 871 (1968).
— CLARK, A.: UrinaryΔ5-pregnene-3β,17α,20α,21-tetrol in 3β-ol-dehydrogenase deficiency. J. clin. Invest. **41**, 1346 (1962).
— EBERLEIN, W.R., GOLDMAN, A.S., NEW, M.: Disorders of adrenal steroid biogenesis. Recent Progr. Hormone Res. **23**, 375 (1967).
— Root, A.W.: The adrenogenital syndrome. New Engl. J. Med. **268**, 1283 (1963).
BRYAN, G.T., KLIMAN, B., BARTTER, F.C.: Impaired aldosterone production in „salt-losing" congenital adrenal hyperplasia. J. clin. Invest. **44**, 957 (1965).
CATHRO, D.M.: The adrenal cortex and medulla. In: HUBBLE, D. (ed.), Paediatric endocrinology, p. 187. Oxford and Edinburgh: Blackwell Sci. Publ. 1969.
— BERTRAND, J., COYLE, M.G.: Antenatal diagnosis of adrenocortical hyperplasia. Lancet **1969 I**, 732.
CHILDS, B., GRUMBACH, M.M., WYK, J.J. VAN: Virilizing adrenal hyperplasia: a genetic and hormonal study. J. clin. Invest. **35**, 213 (1956).
CLEVELAND, W.W., GREEN, O.C., WILKINS, L.: Deaths in congenital adrenal hyperplasia. Pediatrics **29**, 3 (1962).
CONLY, P.W., SANDBERG, D.H., CLEVELAND, W.W.: Steroid metabolism in premature pubarche and virilizing adrenal hyperplasia. J. Pediat. **71**, 506 (1967).
CURTIUS, H.CH., MÜLLER, M.: Gas liquid chromatography of 17-ketosteroids and progesterone metabolites of urine: Comparison of different methods of hydrolysis. J. Chromatogr. **30**, 410 (1967).
DAVID, R.R., BERGADA, C., MIGEON, C.J.: Effect of age on urinary steroid excretion in congenital adrenal hyperplasia. Bull. Johns Hopk. Hosp. **117**, 16 (1965).
DEGENHART, H.J., VISSER, H.K.A., WILMINK, R., CROUGHS, W.: Aldosterone and cortisol secretion rates in infants and children with congenital adrenal hyperplasia suggesting different 21-hydroxylation defects in „salt-losers" and „non-salt-losers". Acta endocr. (Kbh.) **48**, 587 (1965).
— — — FRANKENA, L.: Production and excretion of testosterone in children with congenital adrenal hyperplasia and precocious puberty. Androgens. Proc. 2nd Symposium on Steroid Hormones, Ghent, June 1965, p. 81.
DHOM, G.: Die Nebennierenrinde im Kindesalter. Berlin-Heidelberg-New York: Springer 1965.
EBERLEIN, W.R., BONGIOVANNI, A.M.: Congenital adrenal hyperplasia with hypertension: unusual steroid pattern in blood and urine. J. clin. Endocr. **15**, 1531 (1955).
— — Plasma and urinary corticosteroids in the hypertensive form of congenital adrenal hyperplasia. J. biol. Chem. **223**, 85 (1956).
EARLL, J.M., NEWMAN, S.G., DI RAIMONDO, V.C.: Testicular tumors in congenital adrenocortical hyperplasia. J. Amer. med. Ass. **209**, 937 (1969).
FINKELSTEIN, M.: Pregnanetriolone, an abnormal urinary steroid. In: Methods in hormone research (R.I. DORFMAN, ed.), p. 169. New York-London: Academic Press 1962.
— Enzymatic defects in adrenogenital syndromes. In: L. MARTINI, A. PECILE, ed., Hormonal steroids. Biochemistry, pharmacology and therapeutics. Proc. 1st Intern. Congr. on Hormonal Steroids, p. 625. New York-London: Academic Press 1965.
FRASIER, S.D., HORTON, R., ULSTROM, R.A.: Androgen metabolism in congenital adrenal hyperplasia due to 11β-hydroxylase deficiency. Pediatrics **44**, 201 (1969).
GABRILOVE, J.L., SHARMA, D.C., DORFMAN, R.I.: Adrenocortical 11β-hydroxylase deficiency and virilism first manifest in the adult woman. New Engl. J. Med. **272**, 1189 (1965).
GALAL, O.M., DRAYER, N.M., RUDD, B.T.: Urinary testosterone excretion in children with congenital adrenal hyperplasia. Acta pediat. scand **59**, 137 (1970).
GANDY, H.M., KEUTMAN, E.H., IZZO, A.J.: Characterization of urinary steroids in adrenal hyperplasia: isolation of metabolites of cortisol, compound S, and desoxycorticosterone from a normotensive patient with adrenogenital syndrome. J. clin. Invest. **39**, 364 (1960).
GARDNER, L.I., WYATT, T.C.: Congenital adrenal hyperplasia and rubeola. A study of three fatal cases. Amer. J. Dis. **94**, 452 (1957).
GODARD, C., RIONDEL, A.M., VEYRAT, R., MÉGEVAND, A., MULLER, A.F.: Plasma renin activity and aldosterone secretion in congenital adrenal hyperplasia. Pediatrics **41**, 883 (1968).
GOLDMAN, A.S.: Experimental congenital adrenocortical hyperplasia: persistent postnatal deficiency in activity of 3β-hydroxysteroid-dehydrogenase produced *in utero*. J. clin. Endocr. **27**, 1041 (1967).
GREEN, O.C., MIGEON, C.J., WILKINS, L.: Urinary steroids in hypertensive form of congenital adrenal hyperplasia. J. clin. Endocr. **20**, 929 (1960).
HALL, K., HÖKFELT, B.: Clinical and steroid metabolic studies in four siblings with congenital virilizing adrenal hyperplasia. Acta endocr. (Kbh.) **52**, 535 (1966).
HALPERIN, G., FINKELSTEIN, M.: Biosynthesis of pregnanetriolone and pregnanetriol in congenital adrenal hyperplasia. Acta endocr. (Kbh.) **54**, 439 (1967).
HEDINGER, CHR.: Beidseitige Hodentumoren und kongenitales adrenogenitales Syndrom (Leydig-Zellen oder Nebennierenrinden-Gewebe?). Schweiz. Z. Path Bakt. **17**, 743 (1954).
HIRSCHFELD, A.J., FLESHMAN, J.K.: An unusually high incidence of salt-losing congenital adrenal hyperplasia in the Alaskan Eskimo. J. Pediat. **75**, 492 (1969).
IMAI, M., IGARASHI, Y., SOKABE, H.: Plasma renin activity in congenital virilizing adrenal hyperplasia. Pediatrics **41**, 897 (1968).
JACOBS, D.R.: Natriuretic activity of 16-alpha-hydroxyprogesterone in man. Acta endocr. (Kbh.) **61**, 275 (1969).
JANOSKI, A.H., ROGINSKY, M.S., CHRISTY, N.P., KELLY, W.G.: On the metabolism of 16α-hydroxy-C_{21}-steroids. III. Evidence for high rates of production of 16α-hydroxyprogesterone and 16α-hydroxypregnenolone in the salt-losing form of congenital adrenal hyperplasia. J. clin. Endocr. **29**, 1301 (1969).

JEFFCOATE, T. N. A., DAVIS, J. C., WADE, A. P.: Antenatal diagnosis of adrenocortical hyperplasia. Lancet **1969 I**, 987.

JEUNE, M., BERTRAND, J., CHARRAT, A., LORAS, B., DELAYE, J.: L'hyperplasie surrénale congénitale à forme hypertensive. Pediatrie (Lyon) **18**, 771 (1963).

KINOSHIT, K., ISURUGI, K., KUMAMOTO, Y., TAKAYASU, H.: Gas chromatographic estimation of urinary pregnanetriol, pregnanetriolone and pregnanetetrol in congenital adrenal hyperplasia. J. clin. Endocr. **26**, 1219 (1966).

KOWARSKI, A., RUSSEL, A., MIGEON, C. J.: Aldosterone secretion rate in the hypertensive form of congenital adrenal hyperplasia. J. clin. Endocr. **28**, 1445 (1968).

LIM, N. Y., MIMICA, N., DINGMAN, J. F.: Hyperaldosteronism in hypertensive congenital adrenal hyperplasia. J. clin. Endocr. **29**, 1564 (1969).

MARKS, J. F., FINK, C. W.: Incidence of the salt-losing form of congenital adrenal hyperplasia. Pediatrics **43**, 636 (1969).

MERKATZ, I. R., NEW, M. I., PETERSON, R. E., SEAMAN, M. P.: Prenatal diagnosis of adrenogenital syndrome by amniocentesis. J. Pediat. **75**, 977 (1969).

MIGEON, C. J.: Updating of the treatment of congenital adrenal hyperplasia. J. Pediat. **73**, 805 (1968).

NEW, M.: Antenatal diagnosis of the adrenogenital syndrome. Lancet **1970 I**, 83.

NEW, M. I.: Congenital adrenal hyperplasia. Pediat. Clin. N. Amer. **15**, 395 (1968).

— MILLER, B., PETERSON, R. E.: Aldosterone excretion in normal children and in children with adrenal hyperplasia. J. clin. Invest. **45**, 412 (1966).

— SEAMAN, M. P.: Secretion rates of cortisol and aldosterone precursors in various forms of congenital adrenal hyperplasia. J. clin. Endocr. **30**, 361 (1970).

NICHOLS, J.: Antenatal diagnosis and treatment of the adrenogenital syndrome. Lancet **1970 I**, 83.

— GIBSON, G. G.: Antenatal diagnosis of the adrenogenital syndrome. Lancet **1969 II**, 1068.

PIYARATN, P., ROSAHN, P. D.: Congenital adrenocortical hyperplasia associated with hyperplasia of aberrant (intratesticular) adrenal tissue. J. clin. Endocr. **17**, 1245 (1957).

PRADER, A.: Der Genitalbefund beim Pseudohermaphroditismus femininus des kongenitalen adrenogenitalen Syndroms. Morphologie, Häufigkeit, Entwicklung und Vererbung der verschiedenen Genitalformen. Helv. paediat. Acta **9**, 231 (1954).

— ANDERS, G. J. P. A., HABICH, H.: Zur Genetik des kongenitalen adrenogenitalen Syndroms (virilisierende Nebennierenhyperplasie). Helv. paediat. Acta **17**, 271 (1962).

— SPAHR, O., NEHER, R.: Erhöhte Aldosteronausscheidung beim kongenitalen adrenogenitalen Syndrom. Schweiz. med. Wschr. **85**, 1085 (1965).

RAPPAPORT, R., CORNU, G., ROYER, P.: Statural growth in congenital adrenal hyperplasia treated with hydrocortisone. J. Pediat. **73**, 760 (1968).

REYNOLDS, J. W.: The excretion of two Δ5-3β-OH,16α-hydroxysteroids by patients with congenital adrenal hyperplasia. Pediatrics **36**, 583 (1965).

— ULSTROM, R. A.: Studies of cortisol metabolism in a case of the hypertensive form of congenital adrenal hyperplasia: Demonstration of the absence of 11-β-dehydroxylation. J. clin. Endocr. **23**, 191 (1963).

RIVAROLA, M. A., SAEZ, J. M., MIGEON, C. J.: Studies of androgens in patients with congenital adrenal hyperplasia. J. clin. Endocr. **27**, 624 (1967).

ROSENBLOOM, A. L., SMITH, D. W.: Congenital adrenal hyperplasia. Lancet **1966 I**, 660.

— — Varying expression for salt losing in related patients with congenital adrenal hyperplasia. Pediatrics **38**, 215 (1966).

RUDD, B. T., GALAL, O. M., MCARTHUR, R. G.: A difficulty in confirming 21 hydroxylase deficiency in a neonate with congenital adrenal hyperplasia. Aust. paedit. J. **4**, 38 (1968).

SHARMA, D. C., FORCHIELLI, E., DORFMAN, R. I.: Inhibition of enzymatic steroid 11β-hydroxylation by androgens. J. biol. Chem. **238**, 572 (1963).

SIEBENMANN, R. E.: Die Pathologie der kongenitalen adrenogenitalen Syndrome. Berlin-Heidelberg-New York: Springer 1973.

STEVENS, V. C., GOLDZIEHER, J. W.: Urinary excretion of gonadotropins in congenital adrenal hyperplasia. Pediatrics **41**, 421 (1968).

STÖCKLI, A., KELLER, M.: Kongenitales Adrenogenitalsyndrom und Schwangerschaft. Schweiz. med. Wschr. **99**, 126 (1969).

STOLECKE, H.: Kongenitale Nebennierenrindenhyperplasie mit maximaler Virilisierung (penile Urethra). Kasuistik der Weltliteratur und ein eigener Beitrag. Z. Kinderheilk. **107**, 343 (1970).

STROTT, C. A., YOSHIMI, T., BARDIN, C. W., LIPSETT, M. B.: Blood progesterone, and 17-hydroxyprogesterone levels and production rates in a boy with virilizing congenital adrenal hyperplasia. J. clin. Endocr. **28**, 1085 (1968).

— — LIPSETT, M. B.: Plasma progesterone and 17-hydroxyprogesterone in normal men and children with congenital adrenal hyperplasia. J. clin. Invest. **48**, 930 (1969).

SYMINGTON, TH.: Functional pathology of the adrenal gland. Edinburgh-London: E.S. Livingstone Ltd. 1969.

ZACHMANN, M., VÖLLMIN, J. A., MÜRSET, G., CURTIUS, H. CH., PRADER, A.: Unusual congenital adrenal hyperplasia probably due to 3β-hydroxy steroid dehydrogenase deficiency. Case report and steroid studies. J. clin. Endocr. **30**, 719 (1970).

— — NEW, M. I., CURTIUS, H.-C., PRADER, A.: Congenital adrenal hyperplasia due to deficiency of 11β-hydroxylation of 17α-hydroxylated steroids. J. clin. Endocr. **33**, 501 (1971).

ZAMORA, E., PLATTNER, D., CURTIUS, H.-CH.: Determination of urinary pregnanediol, pregnanetriol and pregnanetriolone in normal children and adults by gas chromatography. Acta endocr. (Kbh.) **62**, 315 (1969).

Acquired Adrenogenital Syndrome in Childhood

ASKARI, H. A., SIEBER, W. H., FETTERMAN, G. H.: Virilizing tumors of the adrenal cortex. Amer. J. Dis. Child. **115**, 445 (1968).

BERNHEIM, M., FRANÇOIS, R.: Les syndromes cortico-surrénaux acquis de l'enfance. Pédiatrie **8**, 357 (1953).

BROOKS, R. V., MATTINGLY, D., MILLS, I. H., PRUNTY, F. T. G.: Postpubertal adrenal virilism with biochemical disturbance of the congenital type of adrenal hyperplasia. Brit. med. J. **1960 I**, 1294.

MAHESH, V. B., GREENBLATT, R. B., CONIFF, R. F.: Adrenal hyperplasia — a case report of delayed onset of the congenital form or an acquired form. J. clin. Endocr. **28**, 619 (1968).

ZURBRÜGG, R. P., JOSS, E., ZUPPINGER, K., KÖNIG, M. P.: Nebennierenrindentumoren im Kindesalter: Tücken und Wertigkeit steroidchemischer Untersuchungen. Schweiz. med. Wschr. **96**, 737 (1966).

Acquired Adrenogenital Syndrome in the Adult

BIRKE, G., FRANKSSON, C., GEMZELL, C. A., MOBERGER, G., PLANTIN, L. O.: Adrenal cortical tumors. A study with special reference to possibilities of correlating histologic appearance with hormonal activity. Acta chir. scand. **117**, 233 (1959).

COHEN, I., THOMAS, R. G.: Adrenal virilizing tumor and pregnancy. S. Afr. med. J. **34**, 46 (1960).

FAGLIA, G., TRAVAGLINI, P., NERI, V., FARRARI, C., GATTINONI, L., ACERBI, L.: Occurrence of a virilizing syndrome with 21-hydroxylase deficiency after pregnancy. J. clin. Endocr. **29**, 1325 (1969).
GABRILOVE, J. L., SHARMA, D. C., DORFMAN, R. I.: Adrenocortical 11β-hydroxylase deficiency and virilism first manifest in the adult woman. New Engl. J. Med. **272**, 1189 (1965).
HEINBECKER, P., O'NEAL, L. W., ACKERMAN, L. V.: Functioning and nonfunctioning adrenal cortical tumours. Surg. Gynec. Obstet. **105**, 21 (1957).
HELD, E.: Adrenogenitales Syndrom bei Carcinom der Nebennierenrinde. Acta endocr. (Kbh.) **17**, 128 (1954).
HUNT, A. B., MCCONAHEY, W. M.: Pregnancy associated with diseases of the adrenal glands. Amer. J. Obstet. Gynec. **66**, 970 (1953).
KENDALL, J. W., SLOOP, P. R.: Dexamethasone-suppressible adrenocortical tumor. New Engl. J. Med. **279**, 532 (1968).
RAPPAPORT, E., GOLDBERG, M. B., GORDON, G. S., HINMAN, F., JR.: Mortality in surgically treated adrenocortical tumors. Postgrad. Med. **11**, 325 (1952).

Idiopathic Hirsutism

BARDIN, C. W., HEMBREE, W. C., LIPSETT, M. B.: Suppression of testosterone and androstenedione production rates with dexamethasone in women with idiopathic hirsutism and polycystic ovaries. J. clin. Endocr. **28**, 1300 (1968).
— LIPSETT, M. B.: Testosterone and androstenedione blood production rates in normal women and women with idiopathic hirsutism or polycystic ovaries. J. clin. Invest. **46**, 891 (1967).
BAULIEU, E. E., MAUVAIS-JARVIS, P., CORPÉCHOT, C.: Steroid studies in a case of Stein-Leventhal syndrome with hirsutism. J. clin. Endocr. **23**, 374 (1963).
CASEY, J. H., NABARRO, J. D. N.: Plasma testosterone in idiopathic hirsutism, and the changes produced by adrenal and ovarian stimulation and suppression. J. clin. Endocr. **27**, 1431 (1967).
DIGNAM, W. J., PION, R. J., LAMB, E. J., SIMMER, H. H.: Plasma androgens in women. II. Patients with polycystic ovaries and hirsutism. Acta endocr. (Kbh.) **45**, 254 (1964).
ETTINGER, B., GOLDFIELD, E. B., BURRILL, K. C., WERDER, K. VON, FORSHAM, P. H.: Plasma testosterone stimulation-suppression dynamics in hirsute women. Amer. J. Med. **54**, 195 (1973).
FORBES, A. P.: Hypertrichosis. New Engl. J. Med. **273**, 602 (1965).
FRANCE, J. T., KNOX, B. S.: Urinary excretion of testosterone and epitestosterone in hirsutism. Acta endocr. (Kbh.) **56**, 177 (1967).
GALLAGHER, T. F., KAPPAS, A., HELLMAN, L., LIPSETT, M. B., PEARSON, O. H., WEST, C. D.: Adrenal hyperfunction in idiopathic hirsutism and the Stein-Leventhal syndrome. J. clin. Invest. **37**, 794 (1958).
GÖBEL, P.: Hirsutismus. Fortschritte auf dem Gebiet der Endokrinologie und der Gastroenterologie. 18. Ärztlicher Fortbildungskurs in Bad Kissingen, p. 13. Stuttgart-New York: F. K. Schattauer 1969 (H. BRÜGEL, Hrsg.).
KAPPAS, A., PEARSON, O. H., WEST, C. D., GALLAGHER, T. F.: A study of idiopathic hirsutism; a transitional adrenal abnormality. J. clin. Endocr. **16**, 517 (1956).
KARL, H. J., RAITH, L.: Der Einfluß von 6-Dehydro-16-Methylenhydrocortison auf die Funktion der Nebennierenrinde bei Frauen mit Hirsutismus. Klin. Wschr. **48**, 347 (1970).
LIPSETT, M. B., RITER, B.: Urinary ketosteroids and pregnanetriol in hirsutism. J. clin. Endocr. **20**, 180 (1960).
LLOYD, C. W., LOBOTSKY, J., SEGRE, E. J., KOBAYASHI, T., TAYMOR, M. L., BATT, R. E.: Plasma testosterone and urinary 17-ketosteroids in women with hirsutism and polycystic ovaries. J. clin. Endocr. **26**, 314 (1966).
— MOSES, A. M., LOBOTSKY, J., KLAIBER, E. L., MARSHALL, L. D., JACOBS, R. D.: Studies of adrenocortical function of women with idiopathic hirsutism; response to 25 units of ACTH. J. clin. Endocr. **23**, 413 (1963).
MAHESH, V. B., GREENBLATT, R. B., AYDAR, C. K., ROY, S., PUEBLA, R. A., ELLEGOOD, J. O.: Urinary steroid excretion patterns in hirsutism. I. Use of adrenal and ovarian suppression tests in the study of hirsutism. J. clin. Endocr. **24**, 1283 (1964).
MÜLLER, S. A.: Hirsutism. Amer. J. Med. **46**, 803 (1969).
NICHOLS, T., NUGENT, C. A., TYLER, F. H.: Glucocorticoid suppression of urinary testosterone excretion in patients with idiopathic hirsutism. J. clin. Endocr. **26**, 79 (1966).
PERLOFF, W. H., CHANNICK, B. J., SUPLICK, B.: Clinical management of idiopathic hirsutism (adrenal virilism). J. Amer. med. Ass. **167**, 2041 (1958).
— JACOBSOHN, G.: Effect of human chorionic gonadotropin on urinary 17-ketosteroids in a patient with Stein-Leventhal syndrome before and after oophorectomy. J. clin. Endocr. **23**, 1177 (1963).
SEGRE, E. J.: Androgens, virilization and the hirsute female. Springfield, Ill.: Ch. C. Thomas 1967.
— KLAIBER, E. L., LOBOTSKY, J., LLOYD, C. W.: Hirsutism and virilizing syndromes. Ann. Rev. Med. **15**, 315 (1964).
WIELAND, R. G., VORYS, N., FOLK, R. L., BESCH, P. K., NERI, A., HAMWI, G. J.: Studies of female hirsutism. Clinical and biochemical evaluation. Amer. J. Med. **41**, 927 (1966).

Adrenal Feminization

BACON, G. E., LOWREY, G. H.: Feminizing adrenal tumor in a six-year-old boy. J. clin. Endocr. **25**, 1403 (1965).
FONTAINE, R., SACREZ, R., KLEIN, M., FRANK, P., LAUSECKER, C., STOLL, G., KAHN, R.: Puberté précoce avec développement des seins chez un garçon porteur d'une tumeur de la surrénale. Arch. franç. Pédiat. **11**, 417 (1954).
GABRILOVE, J. L., SHARMA, D. C., WOTIZ, H. H., DORFMAN, R. I.: Feminizing adrenocortical tumors in the male. A review of 52 cases including a case report. Medicine (Baltimore) **44**, 37 (1965).
LANDAU, R. L., STIMMEL, B. F., HUMPHREYS, E., CLARK, D. E.: Gynecomastia and retarded sexual development resulting from a long-standing estrogen-secreting adrenal tumor. J. clin. Endocr. **14**, 1097 (1954).
MOSIER, H. D., GOODWIN, W. E.: Feminizing adrenal adenoma in a 7-year old boy. Pediatrics **27**, 1016 (1961).
ROSE, L. I., WILLIAMS, G. H., JAGGER, P. I., LAULER, D. P.: Feminizing tumor of the adrenal gland with positive "chorionic-like" gonadotropin test. J. clin. Endocr. **28**, 903 (1968).
SNAITH, A. H.: A case of feminizing adrenal tumor in a girl. J. clin. Endocr. **18**, 318 (1958).
WILKINS, L.: A feminizing adrenal tumor causing gynecomastia in a boy of 5 years contrasted with a virilizing tumor in a 5-year-old girl. Classification of 70 cases of adrenal tumor in children according to their hormonal manifestations and a review of 11 cases of feminizing adrenal tumors in adults. J. clin. Endocr. **8**, 111 (1948).

Assessment of Adrenocortical Function

General

DICZFALUSY, E. (ed.): Steroid assay by protein binding. Acta endocr. (Kbh.), Suppl. **147** (1970).
DORFMAN, R. I. (ed.): Methods in hormone research, vols. 1 and 2. New York: Academic Press 1962.
GRAY, C. H., BACHARACH, A. L. (eds.): Hormones in blood, vols. 1 u. 2, 2nd. ed. New York: Academic Press 1967.

HÜBENER, H. J., STAIB, W.: Biochemie der Nebennierenrinden-Hormone. Stuttgart: Georg Thieme 1965.
MURPHY, B. E. P.: Protein binding and the assay of non-antigenic hormones. Recent Progr. Hormone Res. **25**, 563 (1969).
OERTEL, G. W.: Chemische Bestimmungen von Steroiden im Plasma. Berlin-Göttingen-Heidelberg: Springer 1962.
— Chemische Bestimmungen von Steroiden im menschlichen Harn. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
SUNDERMAN, F. W., SUNDERMAN, F. W., JR. (eds.): Lipids and the steroid hormones in clinical medicine. Philadelphia: Lippincot 1960.

Plasma-ACTH Determination

BERSON, S. A., YALOW, R. S.: Radioimmunoassay of ACTH in plasma. J. clin. Invest. **47**, 2725 (1968).
BESSER, G. M., ORTH, D. N., NICHOLSON, W. E., BYYNY, R. L., ABE, K., WOODHAM, J. P.: Dissociation of the disappearance of bioactive and radioimmunoreactive ACTH from plasma in man. J. clin. Endocr. **32**, 595 (1971).
DEMURA, H., WEST, C. D., NUGENT, C. A., NAKAGAWA, K., TYLER, F. H.: A sensitive radioimmunoassay for plasma ACTH levels. J. clin. Endocr. **26**, 1297 (1966).
LIDDLE, G. W., ISLAND, D., MEADOR, C. K.: Normal and abnormal regulation of corticotropin secretion in man. Recent Progr. Hormone Res. **18**, 125 (1962).
LIPSCOMB, H. S., NELSON, D. H.: A sensitive biologic assay for ACTH. Endocrinology **71**, 13 (1962).
NEY, R. L., SHIMIZU, N., NICHOLSON, W. E., ISLAND, D. P., LIDDLE, G. W.: Correlation of plasma ACTH concentration with adrenocortical response in normal human subjects, surgical patients, and patients with Cushing's disease. J. clin. Invest. **42**, 1669 (1963).
PFEIFFER, E. F., VAUBEL, W. E., RETIENE, K., BERG, D., DITSCHUNEIT, H.: ACTH-Bestimmung mittels Messung des Plasmacorticosterons der mit Dexamethason hypophysenblockierten Ratte. Klin. Wschr. **38**, 980 (1960).
SAYERS, G.: Adrenocorticotrophin. In: Hormones in blood, ed. by C. H. GRAY and A. L. BACHARACH, 2nd ed., vol. 1, p. 169. New York: Academic Press 1967.
YALOW, R. S., GLICK, S. M., ROTH, J., BERSON, S. A.: Radioimmunoassay of human plasma ACTH. J. clin. Endocr. **24**, 1219 (1964).

MSH Determination

ABE, K., ISLAND, D. P., LIDDLE, G. W., FLEISCHER, N., NICHOLSON, W. E.: Radioimmunological evidence for a α-MSH (melanocyte-stimulating hormone) in human pituitary and tumor tissues. J. clin. Endocr. **27**, 46 (1967a).
— NICHOLSON, W. E., LIDDLE, G. W., ISLAND, D. P., ORTH, D. N.: Radioimmunoassay of β-MSH in human plasma and tissues. J. clin. Invest. **46**, 1609 (1967).
HUDSON, B., BENTLEY, G. A.: The biological assay of melanocyte-stimulating hormone. Anstr. J. exp. Biol. **35**, 45 (1957).
LANDGREBE, F. W., WARING, H.: Melanophore expanding activity. In: R. I. DORFMAN, Methods in hormone research. New York: Academic Press 1962.
LERNER, A. B., LEE, T. H.: The melanocyte-stimulating hormones. Vitam. and Horm. **20**, 337 (1962).
SHIZUME, D., LERNER, A. B., FITZPATRICK, T. B.: *In vitro* bioassay for the melanocyte-stimulating hormone. Endocrinology **54**, 533 (1954).
SHIZUME, K., LERNER, A. B.: Determination of melanocyte-stimulating hormone in urine and blood. J. clin. Endocr. **14**, 1491 (1954).
WALKER, J. M.: The melanocyte-stimulating hormone. In: Hormones in blood, ed. by C. H. GRAY and A. L. BACHARACH, 2nd ed., vol. 1, p. 161. New York: Academic Press 1967.

Plasma Renin and Angiotensin Determination

BOUCHER, R., VEYRAT, R., CHAMPLAIN, J. DE, GENEST, J.: New procedures for measurement of human plasma angiotensin and renin activity levels. Canad. med. Ass. J. **90**, 194 (1964).
BOYD, G. W., LANDON, J., PEART, W. S.: Radioimmunoassay for determining plasma-levels of angiotensin II in man. Lancet **1967 II**, 1002.
BROWN, J. J., DAVIES, D. L., LEVER, A. F., ROBERTSON, J. I. S.: Variations in plasma renin concentration in several physiological and pathological states. Canad. med. Ass. J. **90**, 201 (1964).
— — — — TREE, M.: The estimation of renin in human plasma. Biochem. J. **93**, 594 (1964).
CATT, K. J., CAIN, M. C., COGHLAN, J. P.: Measurement of angiotensin in blood. Lancet **1967 II**, 1005.
HABER, E., KOERNER, TH., PAGE, L. B., KLIMAN, B., PURNODE A.: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J. clin. Endocr. **29**, 1349 (1969).
KOTCHEN, T. A., FLAMENBAUM, W., CIRKSENA, W., GAMBLE, W.: Comparison of a biological and an immunological assay for the measurement of plasma renin activity. J. clin. Endocr. **36**, 804 (1973).
PEART, W. S.: The functions for renin and angiotensin. Recent Progr. Hormone Res. **21**, 73 (1965).
VALLOTTON, M. B., PAGE, L. B., HABER, E.: Radioimmunoassay of angiotensin in human plasma. Nature (Lond.) **215**, 714 (1967).
VEYRAT, R., CHAMPLAIN, J. DE, BOUCHER, R., GENEST, J.: Measurement of human arterial renin activity in some physiological and pathological states. Canad. med. Ass. J. **90**, 215 (1964).

Plasma Steroid Determination

Fluorometric Determination of Plasma Corticosteroids

MATTINGLY, D.: A simple fluorimetric method for the estimation of free 11-hydroxycorticoids in human plasma. J. clin. Path. **15**, 374 (1962).
MOOR, P. DE, STEENO, O., RASKIN, M., HENDRIKX, A.: Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. Acta endocr. (Kbh.) **33**, 297 (1960).
SILBER, R. H.: Fluorimetric analysis of corticoids. Meth. biochem. Anal. **14**, 63 (1966).
— BUSH, R. D., OSLAPAS, R.: Practical procedure for estimation of corticosterone or hydrocortisone. Clin. Chem. **4**, 278 (1958).
SWEAT, M.: Adrenocorticosteroids in peripheral and adrenal venous blood of man. J. clin. Endocr. **15**, 1043 (1955).
SWEAT, M. L.: Sulfuric acid-induced fluorescence of corticosteroids. Analyt. Chem. **26**, 773 (1954).

Plasma 17-Hydroxycorticosteroids (Porter-Silber-Chromogens)

PETERSON, R. E., KARRER, A., GUERRA, S. L.: Evaluation of the Silber-Porter procedure for determination of plasma hydrocortisone. Analyt. Chem. **29**, 144 (1957).
PORTER, C. C., SILBER, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids. J. biol. Chem. **185**, 201 (1950).

SILBER, R. H., PORTER, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. J. biol. Chem. **210**, 923 (1954).

Plasma Aldosterone

BRODIE, H., SHIMIZU, N., TAIT, S. A. S., TAIT, J. F.: A method for the measurement of aldosterone in peripheral plasma using ^{3}H-acetic anhydride. J. clin. Endocr. **27**, 997 (1967).

COGHLAN, J. P., SCOGGINS, B. A.: Measurement of aldosterone in peripheral blood of man and sheep. J. clin. Endocr. **27**, 1470 (1967).

KLIMAN, B., PETERSON, R. E.: Double isotope derivative assay of aldosterone in biological extracts. J. biol. Chem. **235**, 1639 (1960).

PETERSON, R. E.: Determination of peripheral plasma aldosterone. In: Aldosterone, ed. by E. E. BAULIEU and P. ROBEL, p. 145. Oxford: Blackwell 1964.

Plasma Androgens

BROWNIE, A. C., MOLEN, H. J. VAN DER, NISHIZAWA, E. E., EIK-NES, K. B.: Determination of testosterone in human peripheral blood using gas-liquid chromatography with electron capture detection. J. clin. Endocr. **24**, 1091 (1964).

GANDY, H. M., MOODY, C. B., PETERSON, R. E.: Androgen levels in ovarian and adrenal venous plasma. Excerpta med. internat. Congr. Series **112**, 223 (1965).

— PETERSON, R. E.: Measurement of testosterone and 17-ketosteroids in plasma by the double isotope dilution derivative method. J. clin. Endocr. **28**, 949 (1968).

HUDSON, B., COGHLAN, J. P., DULMANIS, A., WINTOUR, M., EKKEL, I.: The estimation of testosterone in biological fluids. I. Testosterone in plasma. Aust. J. exp. Biol. med. Sci. **41**, 235 (1963).

RIONDEL, A., TAIT, J. F., GUT, M., TAIT, S. A. S., JOACHIM, E., LITTLE, B.: Estimation of testosterone in human peripheral blood using ^{35}S-thiosemicarbazide. J. clin. Endocr. **23**, 620 (1963).

Direct Eosinophil Count

DUNGER, A.: Eine einfache Methode der Zählung der eosinophilen Leukocyten und der praktische Wert dieser Untersuchung. Münch. med. Wschr. **1910**, 1942.

HENNEMAN, PH., WEXLER, H., WESTENHAVER, M. H.: A comparison of eosin-acetone and phloxine glycol diluents in eosinophil counts. J. Lab. clin. Med. **34**, 1017 (1949).

RANDOLPH, T. G.: Differentiation and enumeration of eosinophils in the counting chamber with a glycol stain; a valuable technique in appraising ACTH dosage. J. Lab. clin. Med. **34**, 1696 (1949).

Urinary Steroid Determination

BREUER, H., NOCKE, W.: Bestimmung von Steroidhormonen. Internist (Berl.) **9**, 63 (1968).

TAMM, J.: Methodik, Fehlerquellen und Deutung der Corticosteroidbestimmungen im Harn. Internist (Berl.) **4**, 559 (1963).

VESTERGAARD, P., LEVERETT, R.: Constancy of creatinine excretion. J. Lab. clin. Med. **51**, 211 (1958).

Urinary 17-Hydroxycorticosteroids

FIEDLER-BEHRENDT, M.: Eine einfache Routine-Bestimmung der 17,21-Dihydroxy-20-Ketosteroide im Urin (nach PETERSON). Röntgen- u. Lab.-Prax. **15**, 132 (1962).

PETERSON, R. E., WYNGAARDEN, J. B., GUERRA, S. L., BRODIE, B. B., BUNIM, J. J.: The physiological disposition and metabolic fate of hydrocortisone in man. J. clin. Invest. **34**, 1779 (1955).

17-Ketogenic Steroids According to NORYMBERSKI *and "Total" 17-Hydroxycorticoids According to* APPLEBY

APPLEBY, J. I., GIBSON, G., NORYMBERSKI, J. K., STUBBS, R. D.: Indirect analysis of corticosteroids. Biochem. J. **60**, 453 (1955).

BORTH, R., LINDER, A., RIONDEL, A.: Urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids in healthy subjects in relation to sex, age, body weight and height. Acta endocr. (Kbh.) **25**, 33 (1957).

NORYMBERSKI, J. K., STUBBS, R. D., WEST, H. F.: Assessment of adrenocortical activity by assay of 17-ketogenic steroids in urine. Lancet **1953I**, 1273.

Free Urinary Cortisol and Free Urinary Corticosteroids

BROOKS, R. V., DUPRÉ, J., COGATE, A. N., MILLS, I. H., PRUNTY, F. T. G.: Appraisal of adrenocortical hyperfunction: Patients with Cushing's syndrome or "non-endocrine" tumors. J. clin. Endocr. **23**, 725 (1963).

ESPINER, E. A.: The relation between free cortisol in urine and "urinary free 11-hydroxycorticosteroids" as measured by fluorescence. J. Endocr. **33**, 223 (1965).

MATTINGLY, D., DENNIS, P. M., PEARSON, J., COPE, C. L.: Rapid screening test for adrenal cortical function. Lancet **1964II**, 1046.

ROSNER, J. M., COS, J. C., BIGLIERI, E. G., HANE, S., FORSHAM, P. H.: Determination of urinary unconjugated cortisol by glass fiber chromatography in the diagnosis of Cushing's syndrome. J. clin. Endocr. **23**, 820 (1963).

Urinary Aldosterone Metabolites, Aldosterone Secretion Rate

KLIMAN, B., PETERSON, R. E.: Double isotope derivative assay of aldosterone in biological extracts. J. biol. Chem. **235**, 1639 (1960).

NEHER, R., WETTSTEIN, A.: Physiochemical estimation of aldosterone in urine. J. clin. Invest. **35**, 800 (1956).

NEW, M. I., MILLER, B., PETERSON, R. E.: Aldosterone excretion in normal children and in children with adrenal hyperplasia. J. clin. Invest. **45**, 412 (1966).

SIEGENTHALER, W. E., PETERSON, R. E., FRIMPTER, G. W.: The renal clearance of aldosterone and its major metabolites. In: Aldosterone (E. E. BAULIEU and P. ROBEL, eds.), p. 51. Oxford: Blackwell 1964.

ULICK, S., LARAGH, J. H., LIEBERMAN, S.: The isolation of a urinary metabolite of aldosterone and its use to measure the rate of secretion of aldosterone by the adrenal cortex of man. Trans. Ass. Amer. Phycns. **71**, 225 (1958).

Urinary 17-Ketosteroids

BORTH, R., LINDER, A., RIONDEL, A.: Urinary excretion of 17-hydroxy-corticosteroids and 17-ketosteroids in healthy subjects, in relation to sex, age, body weight and height. Acta endocr. (Kbh.) **25**, 33 (1957).

DORFMAN, R. I., SHIPLEY, R. A.: Androgens. New York: Wiley 1956.

HAMBURGER, C.: Normal urinary excretion of neutral ketosteroids with special reference to age and sex variations. Acta endocr. (Kbh.) **1**, 19 (1948).
PETERSON, R.E., PIERCE, C.E.: Determination of urinary neutral 17-ketosteroids. In: F.W. SUNDERMAN, and F.W. SUNDERMAN, JR. (eds.), Lipids and the steroid hormones in clinical medicine. Philadelphia: Lippincot 1960.
ZIMMERMANN, W.: Eine Farbreaktion der Sexualhormone und ihre Anwendung zur quantitativen colorimetrischen Bestimmung. Hoppe-Seylers Z. physiol. Chem. **233**, 257 (1935).

Determination of Testosterone and Single Androgenmetabolites in the Urine, Androgen Production Rates

CAMACHO, A.M., MIGEON, C.J.: Studies in origin of testosterone in urine of normal adult subjects and patients with various endocrine disorders. J. clin. Invest. **43**, 1083 (1964).
DULMANIS, A., COGHLAN, J.P., WINTOUR, M., HUDSON, B.: The estimation of testosterone in biological fluids. II. Testosterone in urine. Aust. J. exp. Biol. med. Sci. **43**, 385 (1964).
GURPIDE, E., MANN, J., LIEBERMAN, S.: Analysis of open systems of multiple pools by administration of tracers at constant rate or as single dose as illustrated by problems involving steroid hormones. J. clin. Endocr. **23**, 1155 (1963).
KORENMAN, S.G., WILSON, H., LIPSETT, M.B.: Testosterone production rates in normal adults. J. clin. Invest. **42**, 1753 (1963).
LIPSETT, M.B., KORENMAN, S.G.: Androgen metabolism. J. Amer. med. Ass. **190**, 757 (1964).
PRUNTY, F.T.G.: Androgen metabolism in man, — some current concepts. Brit. med. J. **1966II**, 605.
SCHMIDT, H.: Testosteronausscheidung bei männlichen Personen unter normalen und pathologischen Bedingungen. Acta endocr. (Kbh.), Suppl. 128 zu **58**.
TAIT, J.F.: Review: Use of isotopic steroids for measurement of production rates in vivo. J. clin. Endocr. **23**, 1285 (1963).

Stimulation Tests

ACTH Tests

BIERICH, J.R., SCHÖNBERG, D., ECKLER, E.: Untersuchungen zur Dynamik des Hypophysen-Nebennierenrindensystems. Dtsch. med. Wschr. **87**, 84 (1962).
BIRKE, G., DICZFALUSY, E., PLANTIN, L.O.: Assessment of the functional capacity of the adrenal cortex. I. Establishment of normal values. J. clin. Endocr. **18**, 736 (1958).
— — — Assessment of the functional capacity of the adrenal cortex. II. Clinical applications of the ACTH-test. J. clin. Endocr. **20**, 593 (1960).
CHAKMAKJIAN, Z.H., NELSON, D.H., BETHUNE, J.E.: Adrenocortical failure in panhypopituitarism. J. clin. Endocr. **28**, 259 (1968).
DIXON, P.F., BOOTH, M., BUTLER, J.: In: Hormones in blood, ed. by C.H. GRAY and A.L. BACHARACH, 2nd ed., p. 305. London: Academic Press 1967.
FROESCH, E.R., RENOLD, A.E., THORN, G.W.: Der heutige Stand der Funktionsdiagnostik der Nebennierenrinde. Schweiz. med. Wschr. **89**, 623 (1959).
JENKINS, D., FORSHAM, P.H., LAIDLAW, J.C., REDDY, W.J., THORN, G.W.: Use of ACTH in the diagnosis of adrenal cortex insufficiency. Amer. J. Med. **18**, 3 (1955).
PRUNTY, F.T.G.: Chemistry and treatment of adrenocortical diseases. Springfield: Thomas 1964.
RENOLD, A.E., JENKINS, D., FORSHAM, P.H., THORN, G.W.: The use of intravenous ACTH. A study in quantitative adrenocortical stimulation. J. clin. Endocr. **12**, 763 (1952).

Methopyrapone Test

COPE, C.L., DENNIS, P.M., PEARSON, J.: Some factors determining the adrenal response to metyrapone (SU 4885). Clin. Sci. **30**, 249 (1966).
FROESCH, E.R., LABHART, A., NEHER, R., PRADER, A., ZIEGLER, W.: Diagnostische Anwendung eines neuen Adrenostaticums. Schweiz. med. Wschr. **89**, 623 (1959).
LIDDLE, G.W., ESTEP, H.L., KENDALL, J.W., JR., WILLIAMS, W.C., JR., TOWNES, A.W.: Clinical application of a new test of pituitary reserve. J. clin. Endocr. **19**, 875 (1959).
METCALF, M.G., BEAVEN, D.W.: The metopiron-test of pituitary corticotrophin release. Evaluation of 101 tests. Amer. J. Med. **45**, 176 (1968).

Vasopressin Test

BETHGE, H., BAYER, J.M., WINKELMANN, W.: Diagnosis of Cushing's syndrome. The differentiation between adrenocortical hyperplasia and adrenocortical adenoma by means of Lysin-Vasopressin. Acta endocr. (Kbh.) **60**, 47 (1969).
GWINUP, G.: Test for pituitary function using vasopressin. Lancet **1965II**, 572.
LANDON, J., JAMES, V.H.T., STOKER, D.J.: Plasma-cortisol response to lysine-vasopressin. Comparison with other tests of human pituitary-adrenocortical function. Lancet **1965II**, 1156.
TUCCI, J.R., ESPINER, E.A., JAGGER, P.J., LAULER, D.P., THORN, G.W.: Vasopressin in the evaluation of pituitary-adrenal function. Ann. intern. Med. **69**, 191 (1968).

Insulin and Pyrogen Stimulation Tests

BLISS, E.K., MIGEON, C.J., EIK-NES, K., SANDBERG, A.A., SAMUELS, L.T.: Effects of insulin, histamine, bacterial pyrogen and the antabuse-alcohol reaction upon the levels of 17-hydroxycorticosteroids in the peripheral blood of man. Metabolism **3**, 493 (1954).
ENGEL, E., DEMANET, J.C., BRICHANT, J., RIONDEL, A.M.: Emploi d'un pyrogène comme test de la stimulation neuro-hypophyso-surrénalienne. Helv. med. Acta **25**, 552 (1958).
— LOIZEAU, E., BRICHANT, J., RIONDEL, A.M.: Application clinique d'un test d'hyperstéroidémie provoquée par un pyrogène. Praxis **49**, 417, 446, 461 (1960).
FARMER, T.A., HILL, S.R., PITTMAN, J.A., HEROD, J.W.: The plasma metabolism 17-hydroxycorticosteroid response to corticotropin, SU 4885 and liposaccharide pyrogens. J. clin. Endocr. **21**, 433 (1961).
FROESCH, E.R.: Die Funktion der Nebennierenrinde in der Insulingegenregulation. Schweiz. med. Wschr. **85**, 121 (1955).
JENKINS, J.S., ELKINGTON, S.: Metapyrone and pyrogen in the assessment of pituitary-adrenal function after removal of pituitary adenoma. Lancet **1964II**, 991.
LANDON, J., WYNN, V., JAMES, V.H.T.: The adrenocortical response to insulin-induced hypoglycaemia. J. Endocr. **27**, 183 (1963).

Dexamethasone Suppression Test

BROWN, R.D., VAN LOON, G.R., ORTH, D.N., LIDDLE, G.W.: Cushing's disease with periodic hormonogenesis: One explanation for paradoxical response to dexamethasone. J. clin. Endocr. **36**, 445 (1973).

FRENCH, F. S., MACFIE, J. A., BAGGETT, B., WILLIAMS, T. F., WYK, J. J. VAN: Cushing's syndrome with a paradoxical response to dexamethasone. Amer. J. Med. **47**, 619 (1969).
KATZ, J.: Failure of dexamethasone suppression in adrenal hyperplasia. Arch. intern. Med. **118**, 265 (1966).
JUBIZ, W., MEIKLE, A. W., LEVINSON, R. A., MIZUTANI, S., WEST, CH., TYLER, F. H.: Effect of diphenylhydantoin on the metabolism of dexamethasone. New Engl. J. Med. **283**, 11 (1970).
LIDDLE, G. W.: Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. J. clin. Endocr. **20**, 1539 (1960).
— WILLIAMS, W. C., JR., WALSER, A.: Die Bedeutung von ACTH für die Pathogenese der Cushingschen Erkrankung. Schweiz. med. Wschr. **90**, 1325 (1960).
LINN, J. E., JR., BOWDOIN, B., FARMER, T. A., MEADOR, C. K.: Observations and comments on failure of dexamethasone suppression. New Engl. J. Med. **277**, 403 (1967).
NUGENT, C. A., NICHOLS, T., TYLER, F. H.: Diagnosis of Cushing's syndrome. Single dose dexamethasone suppression test. Arch. intern. Med. **116**, 172 (1965).
PAVLATOS, F. C., SMILO, R. P., FORSHAM, P. H.: A rapid screening test for Cushing's syndrome. J. Amer. med. Ass. **193**, 720 (1965).
ROSE, L. I., WILLIAMS, G. H., JAGGER, P. I., LAULER, D. P., THORN, G. W.: The paradoxical dexamethasone response phenomenon. Metabolism **18**, 369 (1969).
SILVERMAN, W. R., MARNELL, R. T., SHOLITON, L. J., WERK, E. E.: Failure of dexamethasone suppression test to indicate bilateral adrenocortical hyperplasia in Cushing's syndrome. J. clin. Endocr. **23**, 167 (1963).
STROTT, CH. A., NUGENT, CH. A., TYLER, F. H.: Cushing's syndrome caused by bronchial adenomas. Amer. J. Med. **44**, 97 (1968).

Assessment of Origin of Androgens in Hirsutism and Virilization

DAVIS, T. E., LIPSETT, M. B., KORENMAN, S. G.: Suppression of testosterone production by physiologic doses of 2-methyldihydrotestosterone propionate. J. clin. Endocr. **25**, 476 (1965).
DIGNAM, W. J., PION, R. J., LAMB, E. J., SIMMER, H. G.: Plasma androgens in women. II. Patients with polycystic ovaries and hirsutism. Acta endocr. (Kbh.) **45**, 254 (1964).
FORCHIELLI, E., RAO, G. S., SARDA, J. R., GIBREE, N. B., POCHI, P. E., STRAUSS, J. S., DORFMAN, R. I.: Effect of ethinylestradiol on plasma testosterone levels and urinary testosterone excretion in man. Acta endocr. (Kbh.) **50**, 51 (1965).
HORTON, R., TAIT, J. F.: *In vivo* studies of steroid dynamics — androstenedione and testosterone. In: Androgens in normal and pathological conditions, ed. by A. VERMEULEN and D. EXLEY, p. 199. Amsterdam: Excerpta Medica Foundation 1966.
KORENMAN, S. G., KIRSCHNER, M. A., LIPSETT, M. B.: Testosterone production in normal women and women with Stein-Leventhal syndrome or idiopathic hirsutism. J. clin. Endocr. **25**, 798 (1965).
LAMB, E. J., DIGNAM, W. J., PION, R. J., SIMMER, H. H.: Plasma androgens in women. I. Normal and non-hirsute females, oophorectomized and adrenalectomized patients. Acta endocr. (Kbh.) **45**, 243 (1964).
LIPSETT, M. B., KORENMAN, S. G.: Androgen metabolism. J. Amer. med. Ass. **190**, 757 (1964).
— WILSON, H., KIRSCHNER, M. A., KORENMAN, S. G., FISHMAN, L. M., SARFATY, G. A., BARDIN, C. W.: Studies on Leydig cell physiology and pathology: Secretion and metabolism of testosterone. Recent Progr. Hormone Res. **22**, 245 (1966).
MAHESH, V. B., GREENBLATT, R. B.: Steroid secretions of the normal and polycystic ovary. Recent Progr. Hormone Res. **20**, 341 (1964).
SEGRE, E. J.: Androgens, virilization and the hirsute female, Springfield, Ill.: C. C. Thomas 1967.
TAMM, J.: Der Testosteronstoffwechsel beim Menschen. Testosteron im Plasma und Testosteron-Produktionsraten. Dtsch. med. Wschr. **92**, 2037 (1967).

Indirect Assessment of Adrenocortical Function

Water Loading

MOSES, A. M., GABRILOVE, J. L., SOFFER, L. J.: Simplified water loading test in hypoadrenocortism and hypothyroidism. J. clin. Endocr. **18**, 1413 (1958).
SOFFER, L. J., GABRILOVE, J. L.: A simplified water-loading test for the diagnosis of Addison's disease. Metabolism **1**, 504 (1952).

Saliva Electrolytes

LAULER, D. P., HICKLER, R. B., THORN, G. W.: The salivary sodium-potassium ratio. New Engl. J. Med. **267**, 1136 (1962).
PRADER, A., GAUTIER, E., NAEF, D.: Die Na- und K-Konzentration im gemischten Speichel. 1. Der Einfluß der Sekretionsgeschwindigkeit, Stimulations- und Sammelmethode, Geschlecht, Alter, Tageszeit und Salzgehalt der Nahrung. Helv. paediat. Acta **10**, 29 (1955).

Determination of Sodium and Potassium in Sweat and Feces

CONN, J. W., LOUIS, L. H., JOHNSTON, M. W., JOHNSON, B. J.: The electrolyte content of thermal sweat as an index of adrenal cortical function. J. clin. Invest. **27**, 529 (1948).
EDMONDS, C. J., RICHARDS, P.: Measurement of rectal electrical potential difference as an instant screening test for hyperaldosteronism. Lancet **1970 II**, 624.
EMRICH, H. M., ULLRICH, K. J.: Ausscheidung verschiedener Stoffe im Schweiß in Abhängigkeit von der Schweißflußrate. Pflügers Arch. ges. Physiol. **290**, 298 (1966).
GIBSON, L. E., COOKE, R. E.: A test for concentration of electrolytes in sweat in cystic fibrosis of pancreas utilizing pilocarpine by iontonphoresis. Pediatrics **23**, 545 (1959).
GRANDCHAMP, A., SCHERRER, J. R., VEYRAT, R., MULLER, A. F.: I. Measurement of sweat sodium and potassium excretion for evaluation of mineralocorticoid activity in normal subjects. Helv. med. Acta **34**, 367 (1968).
— VEYRAT, R., SCHOLER, D., MULLER, A. F.: II. Measurement of sweat sodium and potassium excretion as a screening test of mineralocorticoid excess in hypertensive patients. Helv. med. Acta **35**, 55 (1969).
HALLER, R. DE, SIEGENTHALER, P., MULLER, A. F.: Influence de l'aldostérone sur la concentration du sodium et du clorure dans la muscoviscidose et chez le sujet normal. Helv. med. Acta **30**, 534 (1963).
LUETSCHER, J. A.: Primary aldosteronism: observations in six cases and review of diagnostic procedures. Medicine (Baltimore) **43**, 437 (1964).
LOCKE, W., TALBOT, N. B., JONES, H. S., WORCHESTER, J.: Studies on the combined use of measurements of sweat electrolyte composition and rate of sweating as an index

of adrenal cortical activity. J. clin. Invest. **30**, 325 (1951).

RICHARDS, P.: Clinical investigation of the effects of adrenal corticosteroid excess on the colon. Lancet **1969 I**, 437.

Total Body Potassium

Clinical uses of whole-body counting. IAEA Wien, 1966.

JOYET, G., BAUDRAZ, A.: Self-attenuation and geometry in single-crystal whole-body spectrometry. Application to total potassium measurement in man. Experientia (Basel) **24**, 865 (1968).

Determination of Exchangeable Potassium and Sodium

CORSA, L., JR., OLNEY, J.M., STEENBURG, R.W., BALL, M.R., MOORE, F.D.: Measurement of exchangeable potassium in man by isotope dilution. J. clin. Invest. **29**, 1280 (1950).

DEMANET, J.C., ENGEL, E., MACH, R.S.: Etude du sodium et potassium échangeables par le Na^{24} et le K^{42} en clinique. Schweiz. med. Wschr. **88**, 1180 (1958).

FORBES, G.B., PERLEY, A.M.: Estimation of total body sodium by isotopic dilution; studies on young adults. J. clin. Invest. **30**, 558 (1951).

Added in Proofs:

BROWN, J.J., CHINN, R.H., FRASER, R., LEVER, A.F., MORTON, J.J., ROBERTSON, J.I.S., TREE, M., WAITE, M.A., PARK, D.M.: Recurrent hyperkalaemia due to selective aldosterone deficiency: Correction by angiotensin infusion. Brit. med. J. **1973 I**, 650.

Editorial: Idiopathic edema. Lancet **1972 I**, 1374.

GILL, J.R., JR., COX, J., DELEA, C.S., BARTTER, F.C.: Idiopathic edema. II. Pathogenesis of edema in patients with hypoalbuminemia. Amer. J. Med. **52**, 452 (1972).

— WALDMANN, T.A., BARTTER, F.C.: Idiopathic edema. I. The occurrence of hypoalbuminemia and abnormal albumin metabolism in women with unexplained edema. Amer. J. Med. **52**, 444 (1972).

KEENAN, J., THOMPSON, J.B., CHAMBERLAIN, M.A., BESSER, G.M.: Prolonged corticotrophic action of a synthetic substituted $^{1-18}$ACTH. Brit. med. J. **1971 III**, 742.

Cushing's Syndrome in Childhood

Editorial: Cushing syndrome in childhood. Lancet **1972 II**, 267.

FONTANELLAZ, H.P.: Das Cushing-Syndrom im Kindesalter. Helv. paediat. Acta **26**, 28 (1971).

LORIDAN, L., SENIOR, B.: Cushing's syndrome in infancy. J. Pediat. **75**, 349 (1969).

MCARTHUR, R.G., CLOUTIER, M.D., HAYLES, A.B., SPRAGUE, R.G.: Cushing's disease in children. Findings in 13 cases. Mayo Clin. Proc. **47**, 318 (1972).

RAITI, S., GRANT, D.B., WILLIAMS, D.I., NEWNS, G.H.: Cushing's syndrome in childhood: postoperative management. Arch. Dis. Childh. **47**, 597 (1972).

RIVAROLA, M.A., MENDILAHARZU, H., DAHL, V., HEINRICH, J.J., SPADA, R.P., MORA, H., BERGADA, C., CULLEN, M.: Pitfalls of functional tests for the establishment of the etiology of cushing's syndrome in childhood and adolescence. J. clin. Endocr. **31**, 254 (1970).

VIII. The Adrenal Medulla

A. LABHART

With Contributions by
CHR. HEDINGER, G. KISTLER, G. TÖNDURY, and W. ZIEGLER

A. Historical Dates

1805 CUVIER differentiated medulla and cortex of the adrenal gland.
1868 FRÄNKEL described the case of an adrenal tumor with pressor crises.
1892 BERDEZ discovered a chromaffin tumor of the adrenal.
1894 Two groups, OLIVER and SCHÄFER and SYZMONOWICZ and CYBULSKI, independently discovered the existence of pressor substances in adrenal extracts.
1898–1905 FÜRTH and ABEL, TAKAMINE and ALDRICH, and STOLZ and DAKIN isolated adrenaline, established its structural formula and synthesized it.
1901 BLUM described adrenal diabetes.
1922 First detailed clinical report of a pheochromocytoma by LABBÉ, TINELLE, and DOUMER.
1927 First successful surgical removal of a pheochromocytoma by MAYO.
1945 HOLTZ, CREDNER, and KRONENBERG discovered noradrenaline.
1957 ARMSTRONG detected vanilmandelic acid as a metabolite of catecholamines.
1957 AXELROD described the inactivation of catecholamines by O-methylation.

B. Embryology and Histology

G. TÖNDURY and G. KISTLER

Embryology. In the human embryo about 11–12 mm in length (early 6th week), neuroblasts which have separated from the primitive ganglia of the celiac plexus come into contact with the medial portions of the adrenocortical primordium (see also Chap. VII, p. 285). At the end of the 6th week, (embryonic length about 14 mm), these sympathetic cells begin to invade the primitive adrenal cortex. As early as the 12th week, they are found to contain small amounts of norepinephrine, and at about 22 weeks the chromaffin reaction (see below) becomes positive. By this time, most of the sympathetic neuroblasts have accumulated in the central portions of the developing gland where they become grouped in cords and clusters of varying size. Immigration of neuroectodermal elements into the fetal adrenal gland continues, however, throughout the prenatal period. The majority of them differentiate into *specific medullary cells*; a small number, however, develop into *sympathetic ganglion cells*, which are scattered singly or in small groups throughout the adrenal medulla.

Histology. The medullary tissue is usually not well preserved in routine necropsy material. When it has been fixed under favorable conditions, however, it is found to be composed of polymorphic epitheloid cells with a clear, rounded nucleus and a large number of minute cytoplasmic granules. In specimens fixed in solutions containing chromium salts, these granules stain specifically brownish yellow. This *chromaffin or pheochromic reaction* is thought to be due to oxidation and polymerization of the *catecholamines* epinephrine and norepinephrine (adrenaline and noradrenaline) within the granules. Since these cytoplasmic organelles disrupt rapidly after death, the cytoplasm of the medullary cells of routine specimens is usually stained diffusely.

The *chromaffin cells* can be histochemically divided into two distinct classes. Those producing *epinephrine* contain large amounts of acid phosphatase and stain intensely with azocarmine. However, these cells do not react with silver salts and are not autofluorescent. In contrast, the medullary cells which synthesize *norepinephrine* are argyrophilic and autofluorescent. They are less well stained with azocarmine and the reaction for acid phosphatase is negative. After double fixation with glutaraldehyde and osmium tetroxide, these two types of pheochromic cells can also be distinguished by electron microscopy. The main mass of the medullary parenchyma consists of cells with moderately electron-dense specific granules. In the rat, these membrane-bound granules have an average diameter of 210 nm and contain epinephrine.

Small clusters of cells, whose granules display a stronger affinity for osmium, are scattered throughout the adrenal medulla, mainly at the periphery of the epitheloid cords. These "dark" cells are thought to synthesize exclusively or predominantly norepinephrine. Their granules are larger than those of the "clear" cells (average diameter in rat 260 nm). In both cell types, the Golgi complex is located in a juxtanuclear position. Its vesicles and cisternae frequently contain electron-dense material which is thought to represent a precursor of the content of the granules. The short cisternae of the rough endoplasmic reticulum are often found to accumulate at the periphery of the cell, together with numerous elongated mitochondria.

The medulla receives blood which has traversed the cortex of the adrenal gland. A network of variably sized capillaries is intimately associated with the chromaffin cells from which it is separated by the corresponding basal laminae and a narrow interstitial space. In their thinnest parts, the endothelial cells display numerous fenestrae. Within the framework of reticular fibers between the epitheloid medullary cords, *sympathetic ganglion cells* occur singly or in small groups. Their pericarya contain moderate amounts of Nissl substance. Numerous preganglionic, sympathetic nerve fibers originating in the celiac plexus penetrate the connective tissue capsule of the adrenal gland and reach the medullary tissue by traversing the cortex. According to STÖHR (1951), every chromaffin cell is in direct contact with nerve endings. When stimulated, the medullary cells seem first to release the content of the granules into the cytoplasmic substance, and the catecholamines then reach the inner surface of the cell membrane by some unexplained pathway, where they can be recognized as small, strongly osmiophilic masses. They are thought to pass through the cell membrane partly in the form of small, cell membrane-bound vesicles (ELFVIN, 1968). In rat, the release of epinephrine from the clear medullary cells can be stimulated by subcutaneous administration of insulin. Six hours after the injection, the granules of the epinephrine-producing cells are found to have increased in size and their content is reduced to a small, electron-dense residual body. In contrast, no morphological changes are observed in the norepinephrine-synthesizing dark cells (MOPPERT, 1966a). In hamster, thirty minutes after an injection of H^3-dopa the concentration of radioactivity in the clear cells is significantly higher than that in the dark cells. Four hours later, however, the activity appears to be evenly distributed over both cell types. The epinephrine-producing cells thus seem to have a more rapid turnover rate, despite the extra methylation step which is necessary to convert norepinephrine to epinephrine. This observation also indicates that the clear cells which store epinephrine are able to perform the whole of the synthesis process of this hormone and that the norepinephrine produced in the dark cells is not transferred to the clear cells for further methylation (ELFVIN, APPELGREN, and ULLBERG, 1966).

C. Biochemistry

1. Chemistry, Site of Formation, Synthesis and Metabolism

Epinephrine and norepinephrine are the hormones of the adrenal medulla. They are called catecholamines because of the pyrocatechol in their basic structure. Dopamine, their immediate precursor, is also a catecholamine and is probably of physiological importance in the metabolism of the brain stem. Norepinephrine is the transmitter substance of the sympathetic system and probably also acts as a transmitter at the synapses of the central nervous system. Catecholamines are formed in the brain, chromaffin tissues, adrenal medulla, and also in the nerve endings of the sympathetic nervous system which are present in almost all tissues. Epinephrine is formed only in chromaffin tissue. Apart from the adrenal medulla, epinephrine is also found in groups of cells lying in the paraganglia scattered in the retroperitoneal tissues. These groups of cells decrease in numbers with increasing age. Catecholamines are formed from tyrosine, the basic amino acid, which circulates in the blood in a concentration of 1–1.5 mg/100 ml and which is taken up by brain, sympathetic and chromaffin cells by an active transport mechanism (Figs. 1 and 2). Tyrosine hydroxylase catalyzes the formation of dopa from tyrosine in the presence of different cofactors and is most probably located in the mitochondria. Amino acid decarboxylase, a nonspecific enzyme found in the free form in the cytoplasm converts dopa into dopamine which is itself oxidized into norepinephrine in the chromaffin granules by the copper-containing enzyme, dopamine-β-oxydase. In the adrenal medulla, most of the norepinephrine leaves the granules again and passes into the cytoplasm. There it is methylated at the N-atom with a methyl group from S-adenosyl methionine by the action of phenyl-ethanol-amine-N-methyltransferase, and is stored again in the granules in the form of epinephrine. The topography of the medulla within the adrenal cortex suggests

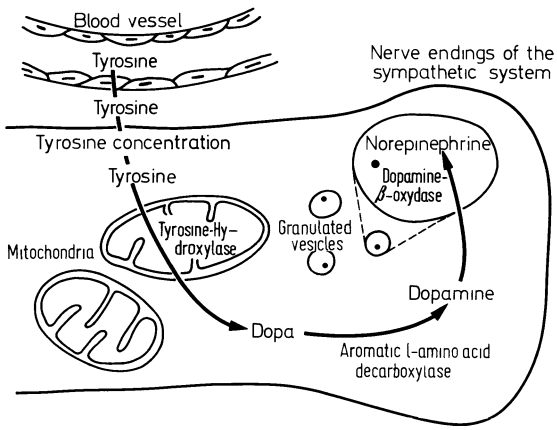


Fig. 1. Intracellular movements of substrates during the biosynthesis of norepinephrine. (After WURTMANN, 1965)

that the enzyme activity of phenyl-ethanolamine-N-methyl-transferase is regulated by dexamethasone or cortisol (WURTMANN, 1966; HARRISON, 1968). The action of glucocorticoids is blocked by puromycin and actinomycin D.

It is improbable that catecholamines other than epinephrine and norepinephrine (isopropyladrenaline, sympathol) play any role as medullary hormones and that the traces of dopamine in the medulla are of any significance. Tyrosine-hydroxylase, and perhaps dopamine-beta-oxydase, regulate the limiting steps in the formation of catecholamines. Dopamine and norepinephrine are formed throughout the sympathetic nervous system. Norepinephrine is also produced in organs with extensive sympathetic innervation, such as the heart. Less epinephrine is excreted in Addison's disease and after bilateral adrenalectomy, whereas norepinephrine excretion remains unchanged. In the human, epinephrine secretion is renormalized years after total adrenalectomy, indicating that inactive chromaffin tissue must be capable of being reactivated (HENKING, 1965). Norepinephrine excretion diminishes after sympathectomy. From this it can be concluded that norepinephrine is formed both in the sympathetic nervous system and in the adrenal medulla, whereas epinephrine is produced only in the medulla and in paraganglia.

2. Storage

Catecholamines are stored within the cells of the adrenal medulla in granules bound to the membrane. These granules are smaller than mitochondria and can be separated from them by centrifugation. About a third of the granules are accounted for by catecholamines, another third by adenosine triphosphate (ATP), and the last third by protein. The molar ratio of catecholamines to ATP is 4 : 1. Catecholamines appear to be bound within the granules in a chemically unaltered but inactive form. Granules similar in appearance but considerably smaller in size and containing only norepinephrine and neither epinephrine nor dopamine are found throughout the entire sympathetic nervous system. The granules form norepinephrine from dopamine, store it, and, in addition, can absorb and inactivate circulating norepinephrine.

3. Release

Catecholamines are inactive as long as they are within the granules. They can be released from the granules by physical, chemical, and neural means. The sympathetic nervous system continuously secretes a small amount of norepinephrine into the blood. Stimulation of the sympathetic nervous system results in the release of much increased amounts. The loss of ATP from the granules is increased by the same factor at the same time. There seem to be two pools of catecholamines in the granules. In one pool, there is a rapid turnover (half-life 2 hours); its catecholamines content is released by the action of tyramine or other sympathomimetic amines and nerve stimulation and can be inactivated by O-methylation. The turnover in the second pool is slow (half-life 24 hours); these catecholamines are not easily mobilized; they are stored in the pool and metabolized by monoamine oxydase (MAO). The adrenal medulla can be regarded as a special type of synapse which conducts the post-ganglionic transmitter substance to the end organ via a humoral instead of a neural route. The liberation of catecholamines which can be induced by insulin hypoglycemia occurs via the central nervous system. In addition, sympathomimetic amines, such as ephedrine and tyramine, cause direct displacement while ATP remains in the granules. Reserpine finally breaks down the granules (see p. 422).

Epinephrine and norepinephrine are not released in direct response to neural impluses in chromaffin tissue and in post-ganglionic fibers of the sympathetic system. Acetylcholine is liberated, which makes the membrane of the fiber permeable to calcium ions. Calcium ions enter the fiber and release noradrenaline from the granule (BURN, 1967). Nicotine and histamine probably also act in the same way as acetylcholine. In the sympathetic nervous system, only norepinephrine is liberated, whereas the medulla releases both epinephrine and norepinephrine into the adrenal vein. It is not known whether the medulla secretes more of

one or the other of the two hormones according to the releasing stimulus. In the human, the adrenal medulla contains between 70 and 90% epinephrine, whereas only traces of dopamine are present.

Certain organs with extensive sympathetic innervation, such as the spleen and myocardium, can absorb considerable amounts of norepinephrine from the blood and store them in the sympathetic nerve endings. Denervation reduces the storage ability and increases the sensitivity of these organs to norepinephrine. The uterus, where catecholamines can be stored outside the nerve-endings, is an exception. Norepinephrine is also formed in the brain and is found in higher concentrations particularly in the region of the brain stem. Parkinson's syndrome is associated with a deficiency of dopamine in the corpus striatum.

4. Transport

It is not yet certain whether catecholamines always circulate in the free form or are also present in the plasma in the bound form. Norepinephrine is stored by thrombocytes *in vitro*, but circulating thrombocytes contain only traces of norepinephrine. The half-life of catecholamines in the blood is about 10 min.

5. Breakdown and Excretion

Inactivation of catecholamines occurs mainly through reabsorption into the granules of the sympathetic nerve endings or breakdown. Catecholamines from the rapid-turnover pool can be inactivated to a minor extent by O-methylation by the enzyme catecholamine-O-methyltransferase, which is found mainly in the liver and kidneys. Normetanephrine and metanephrine are thus produced. These compounds are partly excreted unchanged and partly metabolized by monoamine oxidase to vanilmandelic acid and excreted in this form. Only traces of conjugated catecholamines are excreted. Catecholamines in the slow-turnover pool are first deaminated, and then excreted partly as dihydroxy-mandelic acid and dihydroxyphenyl glycol; the rest are O-methylated and excreted as vanilmandelic acid (VMA).

Only a small percentage of catecholamines (3–7%) appears unchanged in the urine. About 40% is excreted as vanilmandelic acid, and another 40% as normetanephrine or metanephrine, most of these two latter compounds appearing as the glucuronide or the sulfate in the urine. A further 5% is excreted as 3-methoxy-4-hydroxyphenyl glycol. 1% of free and 1% of conjugated 3,4-dihydroxy mandelic acid are excreted as a direct non-methylated product of monoamine oxidase (Fig. 2). 95% of radioactive catecholamines is excreted in the urine within 72 hours. Thus, the VMA excretion is a direct reflection of the total production of catecholamines but is no measure of the norepinephrine and epinephrine acting at the receptors.

D. Pharmacological Influence on Storage, Release, and Inactivation of Catecholamines

Cocaine, *imipramine* and *chlorpromazine* block uptake and binding in the sympathetic nerve endings and thus greatly potentiate the actions of epinephrine and norepinephrine. Release from the active pool is promoted by certain sympathomimetics (*ephedrine*, *amphetamine*). Others act at the same time on α-receptors (phenylephrine, epinine); *tyramine* probably acts via the oxidation product octopamine, a substance which displaces norepinephrine from the active pool.

Reserpine causes mobilization by dissolving the storage granules. Both pools are emptied in the brain and in organs with sympathetic innervation. The catecholamines, however, are deaminated at the same time and thus become inactive.

Release from tissue stores is blocked by bretylium, guanethidine and ganglionic blocking

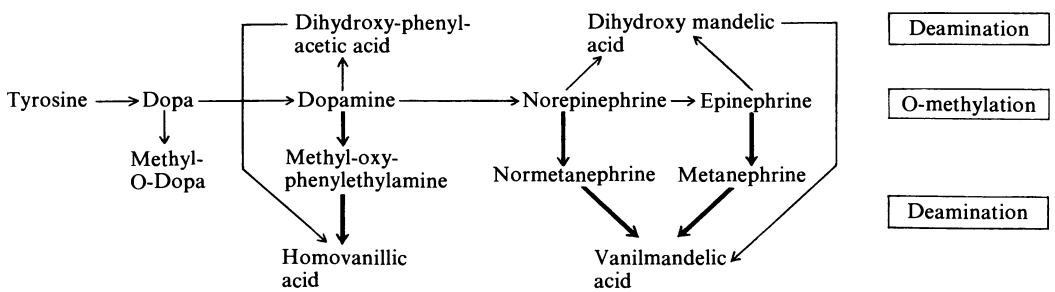


Fig. 2. Biosynthesis and breakdown of catecholamines in man

agents (nicotine), which inhibit particularly the nervously induced liberation. Monoamine oxidase inhibitors interfere with release by their effect on the metabolism of norepinephrine in the nerve cells. False transmitter substances such as metaraminol, α-methyl-dopa, and α-methyl metatyrosine competitively occupy the storage sites in the granules, reducing the norepinephrine content of the tissue. The activity of the sympathetic nervous system is thus diminished. Amino acids cross the blood-brain barrier whereas amines cannot. MAO inhibitors cause accumulation of octopamine formed from tyramine; the former substance can be stored in the granules as a false transmitter and be released by appropriate stimuli.

E. Physiology of the Adrenal Medullary Hormones

1. Actions on Organic Systems

Epinephrine and norepinephrine in physiologic doses have some similar and some antagonistic effects. Unphysiologic overdosage can change these actions.

a) Circulation

Epinephrine increases the pulse rate and cardiac output and thus the systolic blood pressure, whereas it decreases peripheral resistance. Norepinephrine has no effect on the cardiac output, induces bradycardia, and increases peripheral resistance with a rise in systolic and diastolic blood pressure. Unphysiologic doses of epinephrine are required before a rise in peripheral resistance and diastolic blood pressure is produced.

The circulation in the organs is influenced in different ways by the two hormones. Circulation in the skeletal muscle is promoted by epinephrine and inhibited by norepinephrine. Both catecholamines inhibit the blood supply of the splanchnic area and mucosa. Both medullary hormones dilate coronary vessels. The increase in the oxygen demand of the heart caused by epinephrine is greater than would be expected from the increase in pulse rate. The β-receptors of the myocardium (see p. 424) assume positive inotropic actions regulating the ability of the heart to respond to increased functional demands (EPSTEIN, 1966).

b) Musculature

Epinephrine enhances or inhibits the contraction of smooth muscle depending on the location. Epinephrine has a constrictor effect on the dilator muscle of the pupil, on the uterus in the human and cat, and on the erectores pilorum. In contrast, bronchial muscles relax and bronchi dilate; gastrointestinal function is arrested, and the bladder is usually relaxed.

c) Nervous System

Epinephrine, in contrast to norepinephrine, acts on the central nervous system, producing restlessness and a feeling of "uneasy expectancy".

d) Renal Function

Epinephrine and norepinephrine have similar actions on the kidneys. They affect renal hemodynamics and are thus probably an indirect cause of changes in renal function. (Review by WESSON [1961], with tabulated summary of the literature on investigations in man.)

Administration of small doses of norepinephrine (2–4 μg/min) leads to constriction of the vas efferens and reduces renal plasma circulation, whereas filtration initially remains unaltered or even increases slightly due to the rise in pressure (WESSON, 1961). Filtration remaining unchanged in the presence of a decreased plasma flow results in an increase of the filtration fraction. Larger doses (10–14 and 15–44 μg/min) also constrict the vasa afferentia so that filtration is also diminished (PULMAN, 1952; KING, 1956). Total renal vascular resistance increases, particularly that of the afferent part (KING, 1956). Certain influences on electrolyte excretion observed in both man and animals are probably due to hemodynamic changes. Thus, a diminished renal circulation always results in a rise in the reabsorption of sodium (WHITE, 1950; MUELLER, 1951). This is substantiated by the fact that epinephrine and norepinephrine produce a reduction in sodium excretion. Infusion of norepinephrine always gives rise to albuminuria which is due either to the increased hydrostatic pressure in the glomerula or to the ischemia (KING, 1956). The flow of urine remains unchanged with hydration (NICKEL, 1954), but usually decreases with hydropenia (KING, 1956). Diuresis always arises when norepinephrine ceases to act.

2. Action on Carbohydrate and Fat Metabolism

Epinephrine mobilizes energy reserves in the liver, muscle, and adipose tissue. Norepinephrine has this effect only in adipose tissue.

Epinephrine exerts a glycogenolytic action in the liver and muscle by activating phos-

phorylase by the formation of cyclic adenyl monophosphate (see p. 11).

Whereas glucose-6-phosphate liberated in the liver can leave the liver as glucose, it is broken down in the muscle by glycolysis to lactic acid, which is used or released into the blood stream.

Epinephrine and norepinephrine have a lipolytic action on adipose tissue, mobilizing fatty acids and glycerine.

Release of epinephrine or an injection of epinephrine results in a rise in blood sugar, lactic acid, and free fatty acids. Epinephrine and norepinephrine inhibit the release of insulin in response to glucose (see p. 748). Insulin release increases during α-receptor blockade (ILLIG, 1971).

3. General Action on the Organism

During rest and normal activity only norepinephrine is secreted as a transmitter substance in the sympathetic nervous system and for the regulation of blood pressure.

In emergency situations, it is predominantly epinephrine which is released from the adrenal medulla, converting the trophotropic phase into the ergotropic phase, and preparing the organism for immediate action (HESS, 1948). The divergent effects of epinephrine on various organs make sense when BRUECKES' "Notfallreaktion" or CANNON'S "emergency reaction" is kept in mind. The function of organs essential for rapid defense is promoted (eyes, lungs, heart, central nervous system), while the function of organs associated with building (gastrointestinal tract) is arrested. At the same time, necessary fuel, glucose and free fatty acids are set ready for the forthcoming function of the muscles – fight or flight.

4. Regulation of the Secretion of Catecholamines

It is probable but still disputable that the adrenal medulla releases a constant basic amount of catecholamines which cannot be suppressed; denervated adrenal medulla can still secrete small amounts of epinephrine.

In addition, there is a feedback control for catecholamine secretion, which works by neural and humoral routes. Every rise in blood pressure leads to suppression of catecholamine release via the carotid sinus, aortic arch reflex, and the central nervous system. But also, a rise of the epinephrine concentration in the region of the carotid sinus or in the CNS without a rise in blood pressure to a certain level inhibits the secretion of epinephrine. The secretion of catecholamines varies within certain limits in physiologic conditions. In states of shock or hypoglycemia, the inhibitory action can only come into effect with higher concentrations (MALMEJAC, 1964). This mechanism is also controlled by a regulating circle with negative feedback and variable adjustment.

The reader is referred to p. 150 for a discussion on the connection between the adrenal medulla and thyroid function, and for information on the connection between adrenal cortex and medulla to HARRISON (1968), and WURTMAN (1971).

5. Mode of Action and Pharmacological Influence on the Actions

It is assumed that the biological activity of catecholamines is due to interaction of the catecholamine molecules and specific receptors of the end organ. The morphology of these receptors has not yet been defined, nor have they been isolated. The concept is based on indirect pharmacological evidence.

On the basis of pharmacological experience we distinguish between α- and β-receptors. α-Receptors receive mainly the stimulatory actions of the catecholamines (vascular constriction [skin, kidney], conduction in the myocardium, uterine contraction, contraction of the iris dilator) and are inhibited by ergotamine and its derivatives as well as by phentolamine and phenoxybenzamine. Inhibitory actions of catecholamines affect predominantly the β-receptors (vascular dilatation in muscle, uterine relaxation, relaxation of bronchial muscle). They are competitively blocked by propanolol, practolol, alprenolol and oxprenolol (Tables 1 and 2). Propranolol and newer compounds are suitable for clinical use. Blocking of the receptors leads to increased concentrations of catecholamines in the circulation. Thus, β-blockers intensify the action of α-receptors, and α-blockers that of the β-receptors. The chemical structure of β-blocking agents is similar to that of the catecholamines. The action of iso-propylnorepinephrine on β-receptors is particularly marked, and the action of epinephrine is more pronounced than that of norepinephrine.

The receptors of the metabolic processes of glycogenolysis and lipolysis vary to some extent in different species. Lipolysis fails to occur after β-blockade in man. Glycogenolysis in the myocardium and skeletal muscle is always suppressed by β-blocking agents.

As in many other hormone actions, the receptors seem to act via the "second messenger", cyclic AMP. Catecholamines react with the adenylcyclase system, a part of the cell membrane, to form cyclic AMP, which releases

Table 1. Classification and reactions of various end organs to adrenergic stimuli. (After EPSTEIN, 1966)

End organ	Type of receptor	Response
Heart:		
Sinoauricular node	β	Increase in pulserate
Atrioventricular node	β	Increase in conduction velocity and shortening of the functionary, refractory time
Atrium	β	Increased contractability
Ventricle	β	Increased contractability
Smooth muscle:		
Blood vessels of skeletal muscle	1. α 2. β	1. Contraction (constriction) 2. Relaxation (dilatation)
Blood vessels of the skin and mucosa	α	Contraction
Bronchial muscle	β	Relaxation
Gastro-intestinal tract:		
Motility:		
Stomach	β	Decrease
Gut	α and β	Decrease
Sphincters:		
Stomach	α	Contraction
Gut	α	Contraction
Bladder:		
Detrusor	β	Relaxation
Trigonum and sphincter	α	Contraction
Eyes:		
Radial muscle of the iris	α	Contraction (mydriasis)
Ciliary muscle	β	Relaxation (negative accommodation)

Table 2. Functions connected with adrenergic receptors. (From LANGEMANN, after R. P. AHLQUIST, p. 380, Tab. 27-1; in V. A. DRILL: Pharmacology in Medicine, 2nd ed., New York: McGraw-Hill 1958)

α-Receptor; stimulatory actions	β-Receptor; principally inhibitory actions
Vascular constriction (skin, kidney etc.)	Vascular dilatation (skeletal muscle etc.)
Ectopic myocardial excitation	Increase in heart rate and cardiac output
Contraction of splenic capsule	Uterine relaxation (rat, non-gravid cat, human)
Uterine contraction (rabbit, dog, man etc.)	Relaxation of bronchial muscle
Contraction of iris dilator (mydriasis)	
Contraction of nictitating membrane	
Intestinal relaxation	
Pilomotor contraction	
Glycogenolysis, fat mobilization	
? Formation of adrenocorticotropin	
? Ganglionic blocking	
Blocked by usual adrenergic blocking agents	Blocked by dichlorisoproterenol

specific reactions in the cell (SUTHERLAND, 1965, 1968). It has been demonstrated that catecholamines cause an increase in cyclic AMP in the heart, liver, skeletal muscle, and adipose tissue. Cyclic AMP can produce some effects similar to those of the catecholamines. We are now advancing some way towards an understanding of the principles of molecular biology (BELLEAU, 1966).

Although the physiological significance of dopamine is not fully explained, it also exerts pharmacological actions independent of the α- and β-receptors. Thus, the vasodilatatory action of dopamine on mesenteric and renal blood vessels is not inhibited by α- or β-blockers.

α-Adrenergic substances increase peripheral resistance, whereas β-adrenergic substances can be dangerous in shock – in spite of positive inotropic cardiac action – due to the fall in blood pressure. Dopamine in a dosage of 0.5 to 10 μg/min, however, increases cardiac output, renal blood supply, and sodium excretion in healthy subjects and in patients with cardiac insufficiency (MACCANNELL, 1966; MCDONALD, 1964).

F. Hypofunction

Failure of the adrenal medulla has no clinical consequences. Even after adrenalectomy, epinephrine excretion is only temporarily suspended and norepinephrine excretion is not significantly reduced. This suggests that epinephrine must be formed by paraganglionic tissue as well. The reactions of Addisonian patients with primary cortical atrophy are no different than those of patients with Addison's disease due to tuberculosis or after adrenalectomy.

After adrenalectomy, insulin hypoglycemia still results in a rise in plasma epinephrine, albeit delayed and attenuated. Providing there is an adequate glycogen reserve in the liver, the blood sugar shows the same response to insulin in patients with Addison's disease as in healthy subjects.

However two clinical functional disorders are known, one affecting the adrenal medulla and the other the autonomic nervous system.

1. Idiopathic Hypoglycemia in Children (McQuarrie, Zetterström)

Hypoglycemic states with disturbed consciousness and fits arise in children up to the age of 6 years. There are no signs of epinephrine release and the condition is often associated with defects in intelligence and growth. Familial incidence has been described. Epinephrine excretion lies within normal ranges but there is no rise in response to insulin hypoglycemia as in the normal subject, and cortisol levels in the plasma do not usually rise either, whereas the level of growth hormone does (Tietze, 1972). The condition is probably not due to impaired synthesis of epinephrine or a defect in the medulla, but rather to a failure of the hypothalamus to register the hypoglycemia, so that there is no neurally-induced release of epinephrine or neurohormonal release of cortisol. The condition resolves spontaneously after the 6th year of life.

ACTH, glucocorticoids or ephedrine are recommended for treatment. They result in the liberation of epinephrine, but the success of this form of treatment is equivocal.

2. Idiopathic Postural Hypotension (Orthostatic Hypotension, Autonomic Insufficiency)

This condition affects elderly men more than any other group, and consists in the progressive fall of systolic and diastolic blood pressure on rising from the horizontal to the upright position. Sometimes hypoglycemia is an additional symptom, but there is no tachycardia, pallor, or sweating. Other disorders of the sympathetic nervous system, such as anhidrosis and impotence, are often present. These patients become invalids since they become unconscious in the upright position and can only move around in a stooping posture.

In the healthy subject getting up from the horizontal position, norepinephrine is liberated to maintain the blood pressure by counteracting the stagnation of blood in the lower half of the body by constriction of the arterioles and veins. This reflex mechanism is disturbed in idiopathic postural hypotension. The excretion of catecholamines in the urine is sometimes reduced but there is never, as in the normal subject, a distinct rise in plasma norepinephrine on rising from the horizontal to the upright position. The reflex release of epinephrine to hypoglycemia is also absent in these patients. The response to exogenous norepinephrine is not reduced but is even elevated in some cases. The disturbance probably lies in the hypothalamus, in spinal sympathetic centers or in efferent peripheral sympathetic fibers. Recent investigations suggest a connection with degenerative olivo-ponto-cerebellar atrophy (Mark, 1969).

Aldosterone excretion is reduced in these patients, and is only inadequately stimulated by salt withdrawal, ACTH, or angiotensin II. Loss of salt contributes to the circulatory symptoms. This reduction in aldosterone production is more probably a result of chronic hypostimulation than of a disturbance in synthesis in the adrenal cortex (Slaton, 1967).

The differential diagnosis includes vasovagal syncope in which the norepinephrine release is not diminished, but arteriolar constriction is overwhelmed by the strong vagal influence. A *secondary* type of postural hypotension, in contrast to the idiopathic form, is found in association with diabetic neuropathy and other neurological disorders, and with pharmacological blocking of the sympathetic nervous system (guanethidine, ganglion blockers, neuroplegics, monoamine oxidase inhibitors). The secondary form is also found in amyloidosis, adrenocortical insufficiency and hypokalemia, where there is no vasomotor response to norepinephrine. Treatment of idopathic postural hypotension consists of mechanical measures (bandages, elastic stockings, pressure suits of elastic material made to measure). Drugs with vasoconstrictory actions can be used, but they are usually only effective for a short time. Large doses of mineralocorticoids such as 9-α-fluorocortisol appear to work by producing temporary hypervolemia and sensitization of the arterioles.

3. Familial Dysautonomia

(Riley-Day Syndrome)

This is an inherited disorder of the sensory nervous system occurring in the Jewish race. It is also associated with a disturbance in the liberation of catecholamines, but only certain stimuli seem to be involved.

The disorder becomes noticeable during infancy, presenting as dysphagia and recurrent bronchopneumonia. Defective secretion of tears and disturbed motor coordination later become obvious. Orthostatic hypotension is sometimes the predominant feature, but hypertensive crisis and hyperhidrosis can also occur. At the same time, there is hyposensitivity to hypercapnia and hypoxia, and hypalgesia in some parts of the body.

The best way of diagnosing the condition is to inspect the tongue: there are no taste buds and the absence of fundiform papillae is obvious at first sight (DANCIS, 1966).

The orthostatic test reveals that the release of norepinephrine is inadequate in these children. The reaction to insulin is normal since epinephrine release is normal. The adrenal medulla contains an excessive quantity of catecholamines. Excretion of homovanillic acid is increased, whereas excretion of vanilmandelic acid is reduced, suggesting a deficiency of dopamine-β-hydroxylase. In one study, plasma dopamine-β-hydroxylase was in fact found to be decreased, and in one subgroup there was no dopamine-β-hydroxylase at all (WEINSHILBOUM, 1971). Recently, a decrease in norepinephrine synthesis and a shift towards dopamine metabolism has been proved (GOODALL, 1971).

4. Adrenal Medullary Insufficiency in Severe Stress

After exposure to severe stress, such as extensive burns or γ-radiation of the whole body, the excretion of epinephrine and the content of epinephrine in the adrenal medulla may be found to be decreased. It is not known whether these findings are significant within the grave clinical state.

G. Hyperfunction: Pheochromocytoma

1. Incidence

Pheochromocytomas are considered rather uncommon, but data vary widely. Thus, the incidence is variously given as between 1 in 3000 and 1 in 40000 autopsies. The incidence, however, is definitely much higher. The 25274 postmortems performed between 1945 and 1958 at the Pathology Institute of the University of Zurich revealed 23 pheochromocytomas. This gives an incidence of about 0.1% (Table 3). Pheochromocytomas were found in 66 of 12713 patients tested at the Mayo Clinic.

Table 3. Frequency of various tumors arising from the adrenal medulla, obtained in the Pathology Institute of the University of Zurich between 1948 and 1952

Total number of postmortems	8413
Sympathogoniomas	10
Pheochromocytomas	8
Ganglioneuromas	2

Pheochromocytomas make up two-fifths of medullary tumors and are almost as common as sympathogoniomas. SMITHWICK (1950) found 6 pheochromocytomas in 1200 patients (i.e. in 0.5% of cases) in whom he performed sympathectomy for hypertension. Since these tumors appear to be quite common, the possibility of a pheochromocytoma must now be considered in every young patient with hypertension. Pheochromocytomas arise most frequently between the 20th and 50th year but are sometimes present even at birth or appear during advanced age. Women and men are roughly equally affected. Familial incidence has been observed; then pheochromocytomas are multiple in 50% of cases. Neurofibromatosis is also present in 10% of cases. The combination of this disease with hypertension in particular must therefore prompt specific investigation for a pheochromocytoma. Combination with cerebellar angiomas, Hippel-Lindau syndrome, tuberous sclerosis, Sturge-Weber syndrome, Sipple syndrome and brachymetacarpal dwarfism occurs (Chap. XIV, see p. 900). Mixed tumors of the adrenal medulla and cortex occur in few cases. The incidence is sometimes familial and symptoms of Cushing's syndrome may sometimes be associated with these mixed tumors (MATHISON, 1969).

2. Localization

Pheochromocytomas are most often situated in the adrenals (80%), the right adrenal being more commonly affected than the left. Thus, 45% of pheochromocytomas are found in the right and 35% in the left adrenal. In 10% of the cases pheochromocytomas are bilateral, and the remaining 10% involve ganglia outside the adrenals, particularly in the abdominal region and less commonly in the thorax. GRAHAM presents the different localizations on the basis of findings in 204 cases (Table 4).

Table 4. Localization of pheochromocytomas in 213 patients. (After GRAHAM)

The total number of cases mentioned in this summary by GRAHAM does not agree with the 207 observations reported in his text, but in spite of this inaccuracy, a rough picture of the most common localizations can be obtained.

Unilateral pheochromocytomas		Bilateral pheochromocytomas	
Right adrenal	92	Both adrenals	19
Left adrenal	70	2 extra-adrenal tumors	3
Right, lumbar paravertebral	7	1 adrenal tumor on one side and 1 paravertebral lumbar tumor on the other side	1
Left, lumbar paravertebral	5		
Anterior to large abdominal vessels	4	Unknown localization	4
Zuckerkandel's organ	4		
Left, thoracic paravertebral	2		
Celiac ganglion	1		

3. Pathologic Anatomy

In necropsies, *hyperplasia* of the adrenal medulla has been found in patients with chronic circulatory stress and corresponding cardiac changes. Apparently medullary hyperplasia can also be produced by particular strain and hypertension in animal experiments (LIEBEGOTT, 1947). There have also been isolated reports of patients with symptoms similar to those caused by a pheochromocytoma, in whom removal of the hyperplastic medulla brought about a definite cure (MONTALBANO, 1962).

Tumors. Cysts and mesodermal tumors characteristic of the adrenal have already been mentioned briefly under cortical tumors. From the endocrinological point of view, the pheochromocytoma is the most important medullary tumor. Other tumors occurring in the medulla are: neuroblastomas (sympathicogoniomas, sympathicoblastomas, pheochromoblastomas) and ganglioneuromas. These tumors sometimes secrete catecholamines without causing endocrine symptoms (see p. 435).

Sympathicogoniomas are composed of cells corresponding morphologically to the most primitive developmental phase of the series of cells giving rise to differentiated ganglionic cells of the autonomic nervous system and to pheochromocytes. The tumor cells are small, look like lymphocytes, and contain a very dense, chromatin-rich nucleus surrounded by quite a small fringe of cytoplasm. These tumors develop almost exclusively in the adrenals, and 80% of cases occur in children during the first $2^1/_2$ years of life (KARSNER, 1950). They metastasize very rapidly in the liver and skeleton.

Cells of the next developmental phase are termed as sympathoblasts or pheochromoblasts, according to whether they represent the developmental series of sympathetic ganglionic cells or pheochromocytes. Tumors are correspondingly interpreted as *sympathicoblastomas* (sympathoblastomas) or *pheochromoblastomas*. Often, however, tumors of this developmental stage are simply termed as *neuroblastomas*, although this term also covers sympathicogoniomas in English and American usage (SYMINGTON, 1969). Sympathicoblastomas are rather better encapsulated than sympathicogoniomas, and are therefore less malignant. Cell differentiation is further advanced.

Ganglioneuromas and *pheochromocytomas* correspond to the highest phase of development. Some ganglioneuromas are malignant, and are termed as malignant ganglioneuromas or ganglioneuroblastomas. The same is true of pheochromocytomas, but the single term malignant pheochromocytomas has been accepted for these tumors. The term paraganglioma is used for pheochromocytomas arising from extra-adrenal tissue. This term is not completely explicit, since it is also used for tumors of the chemoreceptive organs, which are now also called nonchromaffin paragangliomas or chemodectomas to allow better differentiation.

Pheochromocytomas which cause clinical symptoms are usually at least as large as a cherry. In some cases, however, they have a diameter of several centimeters, and they exceptionally reach a weight of more than 1 kg. Small tumors the size of a pea and a few millimeters at the most in diameter seem to produce no endocrine symptoms. The tumors are usually distinctly encapsulated, especially the larger types, and round in shape. Remains of adrenocortical tissue may also be contained within the capsule. The cut surfaces vary in color between gray and brown and are often darkly pigmented. Cysts containing a brown liquid are sometimes dispersed throughout the tumor. Necroses and hemorrhage are not uncommon. If pheochromocytomas are put in colorless fixatives, such as formalin, the fluid usually assumes a typical dark brown color within a few days, allowing a tentative diagnosis of pheochromocytoma.

Histological section of these tumors reveals cell nests embedded in fibrous tissue with a rich

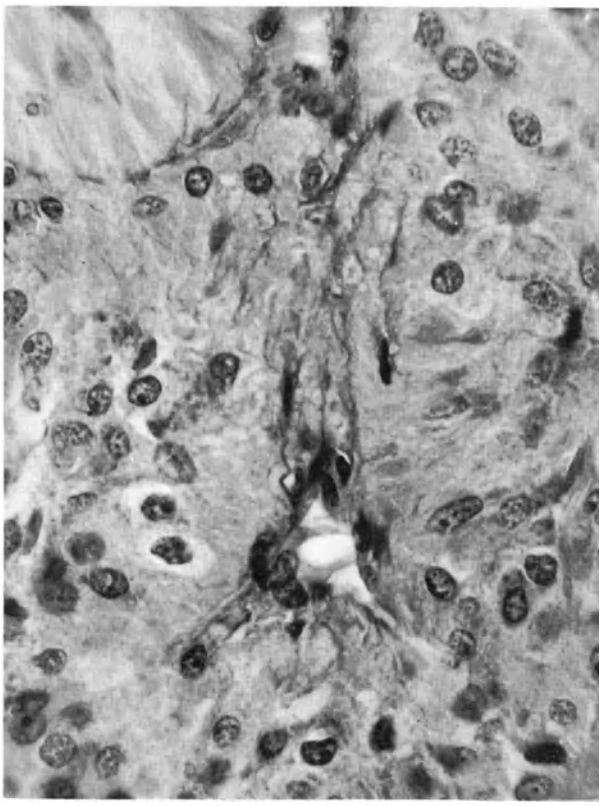


Fig. 3. Pheochromocytoma, MB 12871/55, 47-year-old man. H-E staining; × 450

capillary network, reminiscent of normal medullary tissue. The cells are usually large and polygonal. The outline is usually quite indistinct (Fig. 3). The cytoplasmic borders are acidophilic, slightly granulated, and contain pigment devoid of iron. Multinuclear cells are quite common, but mitoses are infrequent. Occasionally, the cells are better differentiated and possess clear cytoplasmic borders, producing a honeycomb effect, or quite large amounts of fatty substances are deposited in the cytoplasm. A variable number of tumor cells can be impregnated with chromium salts. The cytoplasm and granula take up the stain and turn brownish yellow in color. Often only the cells lying on the periphery of the cell's nests show an affinity for chromium. This affinity is associated with the catecholamine content of the cells.

The morphology of malignant pheochromoblastomas can hardly be differentiated from that of benign tumors, except in cases where metastases can be demonstrated. These malignant tumors are uncommon, their incidence being only one tenth that of the benign tumors at the most. Polymorphia of the tumor cells is not decisive proof of malignancy, because definite nuclear and cellular polymorphia is often found in benign pheochromocytomas. This may be predominantly of a degenerative nature, however. Demonstration of metastases is essential for the diagnosis of malignancy.

Cardiac and vascular changes are responsible for the most important *secondary patho-anatomical symptoms*. Thus, hypertension leads to such signs as cardiac hypertrophy, vascular changes such as arteriosclerosis, arteriolosclerosis, and even arteriolonecrosis. Other features worth mentioning are myocardial changes such as myocarditis and myocardial fibrosis, which are interpreted as direct results of the endocrine activity of the pheochromocytoma. Renal infarcts have been described as characteristic by ZOLLINGER (1959). For tumor combinations with neurofibromatosis or thyroid tumors see Chap. VI, p. 232.

4. Clinical Picture and Symptoms

The symptom complex of pheochromocytomas includes: 1. paroxysmal hypertension, 2. persistent hypertension, and 3. metabolic symptoms, such as hypermetabolism, hyperglycemia and

glucosuria, and occasionally diabetes mellitus and hyperlipolysis.

Symptoms can occur in any combination. They may arise singly or sometimes be completely absent. In some cases hypertension leads to cerebral vascular accidents and the elevated O_2 requirement of the heart to myocardial infarctions. Unusual symptoms due to the particular localization (ureteral occlusion, renal arterial stenosis, abdominal pains) have frequently been described.

The symptoms allow only limited conclusions about the nature of the hormones being produced to excess, although tachycardia, increased metabolic rate and glucosuria are characteristic of overproduction of epinephrine. Extreme overdosage of norepinephrine can also produce these symptoms, however.

a) Paroxysmal Hypertension

Pheochromocytoma with paroxysmal hypertension is characterized by typical attacks which allow a fairly definite diagnosis.

The sudden acute rise in systolic blood pressure to 200–300 mm Hg, which is sometimes preceded by a sort of aura with blacking out, nausea, and pains in the extremities, is associated with signs and symptoms caused by the release of epinephrine; tachycardia with thready pulse, pallor, outbreak of sweating, tremor, anxiety and palpitations, headache, visual disturbances, pain and pressure in the upper abdomen, nausea, vomiting and dyspnea, paresthesia, diarrhea occurs in some cases, and occasionally epileptic seizures.

During an attack, leukocytosis of between 10000 and 30000 with lymphocytosis, glucosuria, and hyperglycemia can be detected. Although the skin is cool to the touch, body temperature, particularly rectal temperature, is raised due to stagnation of heat. Peripheral cyanosis and a condition reminiscent of Raynaud's phenomenon sometimes develop. The thyroid gland may become swollen. In severe cases, circulatory failure with pulmonary edema finally arises, with advanced cyanosis and collapse. Rhythmic disturbances such as sinus arrhythmia, tachycardia and bradycardia, nodal rhythm, ventricular and supraventricular extrasystoles and ventricular fibrillation sometimes occur. Active "catechol myocarditis" characterized morphologically by myocardial necroses and histocytic and lymphocytic infiltrations are thought to be found at autopsy in 50% of patients with pheochromocytoma (VAN VLIET, 1966). At the end of the attack the blood pressure usually returns rapidly to the original value, the patient becomes flushed, and the profuse sweating and secretion of saliva may persist. Patients feel tired and spent for a long time afterwards. In contrast to the severely disturbed vascular system and carbohydrate and fat metabolism, the influence of the pheochromocytoma on the kidneys is usually discrete, though it is occasionally severe. Very often, neither functional nor morphological changes can be found in the kidneys (GREEN, 1946; SACK, 1963). Frequently, a discrete pathologic finding is noted at urinalysis, such as albuminuria, and occasionally microhematuria in association with paroxysmal or persistent hypertension. Albuminuria, microhematuria and cylindruria arise intermittently, and simulate nephritis (FERTIG, 1951). A pheochromocytoma must be suspected in every case of "paroxysmal" nephritis. Occasionally inhibition of diuresis with azotemia ranging to full anuria is seen during an attack (BAIRD, 1954; ZOLLINGER, 1959). It is partly explained by the constriction of the vasa afferentia and reduced renal plasma flow. Hyposthenuria has been observed. Termination of an attack is always followed by a flood of up to 5–7 liters of urine. Hyposthenuria and polyuria caused by the action of epinephrine may be due to the decreased renal medullary hypertonicity resulting from an elevated medullary blood flow, which, in contrast to cortical circulation, increases with a rise in arterial pressure (BUCHBORN, 1961). Electrolyte disorders associated with a pheochromocytoma are only rarely observed clinically, although from acute experiments the opposite would be expected.

Attacks are induced by exertion, postural changes causing compression of the adrenal area, straining on defecation and micturition, and also by excitement, fear, and anger, as well as by smoking. Iatrogenic attacks can be induced during general anesthesia and during the investigation of anacidity with histamine. Sudden collapse under such conditions should always suggest a pheochromocytoma. Attacks can, however, also arise spontaneously during absolute rest and even during sleep. The attacks usually last between 15 min and 2 hours, but can also be over in a few minutes or persist for a whole day. Initially, attacks can arise every few weeks or months. They usually become more frequent and severe until they finally occur daily. Sometimes there are intervals of several years between attacks.

b) Persistent Hypertension

Persistent hypertension is more common than paroxysmal hypertension in pheochromocytomas. The symptoms it causes are in no way

different from those of benign or malignant hypertension.

The signs and symptoms listed below can be regarded as indications for investigation of patients with hypertension for pheochromocytomas:

1. Attacks of headaches, palpitations, nervousness, thoracic or abdominal pains, tremor, hypertension, persistent or frequent excessive sweating.
2. Leanness with more than 10% underweight or a body weight of less than 48 kg.
3. Age of 35 years or less.
4. Basal metabolic rate 20% above normal in the absence of hyperthyroidism.
5. Duration of hypertension of less than 2 years.
6. Fundus changes, grade III or IV or severe forms of grade II.
7. Paradoxical reaction of blood pressure to ganglion blockers.
8. Blood pressure reaction at the onset of general anesthesia.

Pheochromocytomas must be suspected particularly in hypertension affecting young patients. About 100 cases of hypertension due to a pheochromocytoma have been reported in children. Hypertension in these cases was predominantly of the persistent type. Growth is arrested. Blood pressure is always considerably elevated, particularly the diastolic level (upper normal level of systolic pressure in children = 100 + twice age in years). Spastic changes are usually found in the ocular fundus, and papilledema or hemorrhage sometimes occurs.

In adults, the three cardinal symptoms of headaches, sweating, and palpitations are seldom absent. However, the suspicion of a pheochromocytoma can also be evoked by a considerably elevated basal metabolic rate or severe sweating and glycosuria in the presence of hypertension with concomitant bradycardia (so-called "H-triad" (HOWARD): hyperglycemia, hypertension, hypermetabolism without hyperthyroidism). Repeated estimations of the basal metabolic rate and blood glucose are indicated in such cases. Impotence due to impaired autonomic equilibrium can occur.

In cases of pheochromocytoma with persistent hypertension of long duration, the plasma volume can be considerably diminished due to persistent vasoconstriction (up to 20%), so that removal of the tumor may be followed by an immediate dangerous fall in blood pressure with oliguria, anuria and myocardial ischemia if preoperative treatment is inadequate (ZIEGLER, 1966; BRUNJES, 1960). Plasma renin is elevated accordingly (see Chap. XV). Vascular complications are also possible, with changes in the *fundus oculi*, impaired renal function, and renal infarct (see p. 429). The hypertension may be permanent in such cases, susceptible to reduction but no longer to correction by removal of the pheochromocytoma. In some patients, paroxysmal attacks are superimposed on the persistent form, and are associated with all the characteristic features. Not infrequently, the disorder begins with paroxysmal hypertension which progresses to the persistent form. As in essential hypertension, death results from apoplexy, cardiac failure, or uremia. Finally, in some cases the hypertension is extremely labile, varying from one minute to the next.

Table 5. Frequency of pheochromocytoma symptoms. [After GIFFORD: Mayo Clin. Proc. **39**, 281 (1964)]

Symptoms	Paroxysmal form (%)	Persistent form (%)
Headaches	92	72
Excessive sweating	65	70
Palpitations with or without tachycardia	73	51
Pallor, particularly in face	60	28
Nervousness or anxiety	60	28
Tremor	51	26
Nausea with or without vomiting	43	26
Weakness, exhaustion or tiredness	38	15
Thoracic pains	32	13
Abdominal pains	16	15
Visual disorders	3	21
Weight loss over 10%	14	15
Raynaud's phenomenon	8	3
Dyspnea	11	18
Constipation	0	13
Grand mal	5	3
Sensation of heat or hot flushes or both	11	8
Bradycardia, perceived by patients	8	3
Intolerance to heat	3	8
Giddiness or confusion	11	3
Paresthesia or pains in the arms	11	0

If the hypertension persists after removal of a pheochromocytoma the established hypertension shouldnt simply be accepted as a result of arteriolosclerotic changes. A careful search must first be made for a second pheochromocytoma. This is best done actually during the operation, by means of the phentolamine test. Secondly, renal infarcts and cortical atrophy are not uncommon after pheochromocytoma operations, due to lesions of the renal vessels (HARRISON, 1958; SACK, 1963). An infarcted kidney can lead to renal hypertension. In a few cases, pheochromocytomas have caused mechanical inhibition of renal blood supply, inducing a Goldblatt mechanism (ROSENHEIM, 1963; WEIDMANN, 1969).

In general, however, renal dysfunction is completely reversible after successful removal of the pheochromocytoma.

Table 6. Laboratory findings in pheochromocytoma. [After GIFFORD: Mayo Clin. Proc. **39**, 281 (1964)]

Findings	Paroxysmal form (%)	Persistent form (%)
Ocular fundus		
Negative	51	5
Hypertension I	26	16
Hypertension II	23	26
Hypertension III	0	16
Hypertension IV	0	37
Fasting blood sugar		
Below 90 mg% (autoanalyzer)	44	34
90–109 mg%	32	31
Over 109 mg%	24	35
Basal metabolic rate		
Below +10%	56	9
+10 to +19%	16	15
+20 to +49%	28	52
Over +50%	0	24
Albuminuria	35	64
Blood urea		
less than 40 mg%	81	76
40–59 mg%	16	19
60 mg%	3	5

In contrast to the situation in the paroxysmal form, clinical observation alone is no help, and the doctor is dependent on laboratory examinations. There is, however, one feature characteristic of patients with pheochromocytomas; they are said always to be thin due to the lipolytic action of catecholamines, and obesity is said to exclude the disease. Unusual clinical symptoms, such as paroxysmal abdominal pains and gastrointestinal hemorrhages, have been described. Circulatory disorders similar to those associated with Raynaud's disease also occur with the persistent hypertension.

An impaired metabolism of carbohydrates similar to that in diabetes mellitus is found in between one third and one quarter of patients with pheochromocytomas. This condition is characterized by reduced glucose tolerance, fasting hyperglycemia and glucosuria. Ketosis, however, is rare. This feature was previously explained by the increased glycogenolysis, but it is currently thought to be due primarily to the inhibiting effect of epinephrine and norepinephrine on the release of insulin in response to glucose (see p. 748). At the same time, there is also hyposensitivity to insulin. Free fatty acids and glycerine in the plasma are thought to be elevated, but we have not always been able to confirm this in our own investigations. Insulin release is normal again shortly after the operation; in contrast, glucose tolerance takes some months to return to normal (SPERGEL, 1968; WILBER, 1966).

c) Hypotension

Occasionally, the symptoms experienced are attacks of *hypotension* as low as 70/50 mm Hg, with tachycardia, pallor, sweating, leukocytosis, and glucosuria. It is possible that these attacks are preceded by rises in blood pressure of very short duration, which are completely overlooked. Low doses of epinephrine can have a hypotensive action, particularly if given repeatedly. Attacks can become manifest in tachycardia and outbreaks of sweating only, with no change in blood pressure.

In the type with persistent hypertension, there is sometimes a tendency to orthostatic fall in blood pressure, particularly if patients are being treated with phentolamine or phenoxybenzamine. The blood pressure falls to normal or hypotensive levels in the upright position. The additional release of norepinephrine, which normally occurs on standing, obviously no longer functions in such cases, or the adrenolytic drugs led to hypovolemia (see p. 436).

d) Special Forms and Combinations with Other Syndromes

α) Malignant Pheochromocytomas

The malignant pheochromocytoma with invasive growth and metastases is uncommon. Aberrant adrenal tissue in the retroperitoneal space must be excluded. Recurrences usually arise within one year following the primary operation. Life expectancy after malignancy has been confirmed is usually less than 3 years but can even be as long as 8 years. In cases where the course is slow, periodic operations to remove metastases may be worthwhile, since symptom-free intervals of several years may be achieved in this way. Local recurrences are particularly common. The incidence of metastases in the liver, lymph nodes, lungs, and skeleton is roughly the same. Forms where the course of the disorder is unusually slow – we are currently following a case which has extended over 40 years – indicate malignant degeneration of an originally benign pheochromocytoma. Elevated urinary excretion of dopamine and its metabolites is an indication of malignancy of a pheochromocytoma.

Palliative medical treatment is justified in inoperable cases (see p. 436). This form of treatment can at least abolish symptoms caused by the overproduction of catecholamines.

It is advisable to try cytostatics. Temporary success can be achieved with alkylated substances (cyclophosphamide).

A few types of tumors of neuroectodermal origin, such as neuroblastomas and chemodectomas, can be detected in the absence of endocrine symptoms from the increased excretion of catecholamines, and particularly from the presence of abnormal metabolites in the urine.

β) Familial Pheochromocytomas, Combinations with Other Syndromes
(see also Chap. XVIII, Pluriglandular Syndromes)

Familial pheochromocytomas are inherited on an autosomal dominant gene. They are often multiple and ectopic, and in children they are more common than nonfamilial pheochromocytomas. Pheochromocytomas also occur in association with RECKLINGHAUSEN'S neurofibromatosis (SCHLEGEL, 1960), with ganglioneuromas (STREIT, 1967), the Hippel-Lindau syndrome, and brachymetacarpal dwarfism (NAGANT DE DEUXCHAISNES, 1960) (see Chap. XIV).

γ) Association with Medullary Carcinoma of the Thyroid Gland

Over 30 cases of medullary carcinoma of the thyroid gland together with pheochromocytomas have been reported. The features of these patients are very characteristic, and they are all similar in appearance. The face with the pouting lips is reminiscent sometimes of acromegaly and sometimes of the Marfan syndrome. Neuromas of the eyelids and tongue are characteristic (see Chap. VI, p. 233). This disorder appears to have an autosomal dominant mode of inheritance. It is possible that the same gene is involved as causes familial pheochromocytomas. The combination with neurofibromatosis indicates an ectodermal disorder of the anlage, and the medullary carcinoma of the thyroid gland, like the chromaffin tissue, is regarded as a derivative of the ganglionic ridge ("familial chromaffinomatosis", LJUNGBERG, 1967). It is now known, however, that medullary carcinoma arises from parafollicular cells of the thyroid gland and that these cells originate ultimately in the neural crest. Combinations with parathyroid hyperplasia and Cushing's syndrome are discussed in Chap. XVIII (p. 1009).

5. Diagnosis of Pheochromocytoma

W. ZIEGLER

The diversity of the clinical picture is the main reason for the frequent long delays in arriving at the diagnosis. If a pheochromocytoma is suspected on clinical grounds the diagnosis must be confirmed by means of all diagnostic aids. Exploratory laparatomy, however, should never be considered for this purpose.

Case reviews from the Mayo Clinic show that the diagnosis is suspected on the basis of clinical observation in 9 out of 10 cases (GIFFORD, 1964).

Paroxysmal changes in blood pressure with all the accompanying features in a normotensive person are much more impressive and easier to detect than persistent hypertension, where the superimposed variations in blood pressure are often hardly measurable. In every case, however, the diagnosis must be confirmed before surgery by increased formation of catecholamines revealed by biochemical analyses of the urine and clinical pharmacological tests.

Pharmacological tests either provoke the release of catecholamines (induction of a blood pressure crisis) or block the effect of high plasma catecholamine levels (induction of a fall in blood pressure). They are valuable diagnostic aids which can be carried out without special laboratories if the doctor is prepared to spend an adequate amount of time on them, and observes certain precautions. The diagnosis, however, must always be confirmed by demonstration of an increase of catecholamines or their metabolites in the urine. The importance of previous pharmacological tests is decreasing with the widespread availability of simple methods of urinalysis. After confirmation of the diagnosis, the tumor must be localized as exactly as possible. It is advisable to measure blood volume with a view to the preoperative preparation.

a) Pharmacological Tests

The tests can only be carried out if the following conditions are fulfilled:

There should be no signs of renal or cardiac insufficiency. A history of myocardial infarction or apoplexy contraindicates these tests.

All drugs acting on the central nervous system, such as alcohol, hypnotics, sedatives, opiates, and psychotropic drugs, can distort results, as can drugs acting on synapses and nerve endings. All sympatho- and parasympathomimetics and their corresponding blocking agents, and other substances which stimulate or block the ganglia thus distort results.

Antihypertensives such as the veratrum alkaloids can also affect the test results. Reserpine (Serpasil) and α-methyl-Dopa (Aldomet) in particular must be withdrawn for at least 10 days before the test. If clinically possible, drug therapy should be completely withdrawn 48 hours before these tests are carried out.

A blocking test can be false-positive due to sedatives, for example, and antihypertensives can cause either false-negative or false-positive results.

The test itself should be performed in a separate room. It is essential that the patient rests in the supine position for at least 30 minutes before the test and that the blood pressure and pulse rate are measured until reliable base-line values are obtained.

α) Provocation Tests

These are based on depletion of the catecholamine stores of the tumor and the sympathetic nerve endings, thus inducing paroxysmal hypertension.

1. Cold Pressure Test. This test should always be performed immediately before the provocation tests, since conclusions are only possible when results of the two examinations are compared.

The cold pressure test is contraindicated in cases where the diastolic blood pressure is higher than 150 mm Hg. In the healthy subject, the short-acting stimulus usually produces a rise in blood pressure, the degree of this rise indicating the circulatory lability. In the patient with vegetative stigmata it will usually exceed the rise caused by histamine.

Method: The patient immerses one hand in a water bath with ice cubes (4 °C) for one minute. Pulse and blood pressure are measured after 30 and 60 seconds.

Interpretation: A considerable rise of the blood pressure indicates vegetative dystonia or possibly even labile essential hypertension.

2. Histamine Test. Depending on the age of the patients, this test should not be applied when blood pressure values exceed 150 or 170/110 mm Hg. Histamine leads initially to vasodilatation and a fall in blood pressure, followed immediately by a release of catecholamines from the stores.

Method: 0.05 mg (1/20 mg) of histamine in 0.5 ml physiological saline is given by rapid i.v. injection. The blood pressure is then measured every minute for the next 15 min. A first measurement after 30 sec detects the initial fall in blood pressure. Phentolamine (Regitin) and norepinephrine (Arterenol) should be prepared and be kept ready for immediate use to avert any threatened blood pressure crisis or hypotension. It is best to keep an intravenous route open throughout the test by maintaining a saline drip.

Interpretation: A rise in blood pressure to 60/40 mm Hg above the initial levels within 1 to 4 min after the injection, together with the appearance of symptoms typical of a hypertensive attack, is considered as a positive result. The blood pressure values obtained in the cold pressure test should be exceeded. If these conditions are fulfilled, the attack should be abolished by means of intravenous phentolamine. False-negative and false-positive results are obtained in 5% of tests.

3. Tyramine Test. Tyramine mobilizes catecholamines from the granules of the sympathetic nerve endings. It causes no unpleasant side effects like histamine does (headache, sensation of heat).

Method: At the onset, physiological saline is given by i.v. injection while blood pressure is monitored continuously. Tyramine is then injected in doses increasing from 250 to 500 to 1000 μg (1 mg). The blood pressure should be measured every minute. An interval of 15 min should elapse between injections.

Interpretation: A rise of the systolic pressure by at least 20 mm Hg within 1–2 min suggests a pheochromocytoma. False-positive results are obtained in 5% of the tests, whereas results are false-negative in 30%.

4. Glucagon Test. This test is also based on the release of catecholamines from the adrenal medulla and the tumor. As in the histamine test, it is advisable to compare the results with those obtained in the cold pressure test.

Method: At least 0.5 mg of glucagon, but usually 1.0 mg, is administered to the fasting patient by rapid i.v. injection. The blood pressure is subsequently measured every 30 sec for 5 min and then every minute for a further 10 min. Phentolamine and norepinephrine must be kept ready during this test also.

Interpretation: Criteria for a positive result are similar to those in the histamine test, although side effects are much more uncommon and less pronounced than with histamine. False-positive results have not been reported so far. False-negative results, however, have frequently been observed.

β) Blocking Tests

These tests are based on the inactivation of circulating catecholamines by means of α-

receptor blockade. The decrease in peripheral resistance causes a momentary fall in blood pressure. Phentolamine is the only α-blocking agent which has proved satisfactory.

Phentolamine (Regitin) Test. This test should not be performed when blood pressure is below 160/110 mm Hg.

Method: Access to an intravenous route of administration throughout the investigation is recommended, and is best achieved by setting up a saline drip. After i.v. injection of 5 mg Regitin (half an ampoule) (3 mg/m^2 in children) over 5–10 sec the blood pressure is measured every minute. There is a risk of inducing circulatory collapse, so that norepinephrine must always be kept ready for immediate injection.

Interpretation: A fall in blood pressure of 40/25 mm Hg below the initial levels within 5 minutes is considered a positive result. The initial levels should not be recovered until 5–15 min after the injection. False-positive results are obtained in 2–3% of the tests, and if the condition cited above is not observed 20% of the tests can be expected to give false-positive results. False-negative results are uncommon.

b) Biochemical Investigations

Demonstration of increased excretion of catecholamines and their metabolites in the urine is essential for confirmation of the diagnosis. Nonspecific quick tests should be avoided since they often give false-positive results.

Method of Urine Collection. The patient must refrain from consuming bananas, citrus fruits, nuts, tea, coffee, and vanilla for two days before collecting the urine. A 24-hour specimen of urine is then collected during the second day of diet. Acid is added to the urine (1 ml of concentrated perchloric acid to 100 ml of urine; concentrated hydrochloric acid may be used instead). The urine must be protected from heat and light. If the 24-hour specimen is correctly measured only 200 ml urine are required for the analysis.

Numerous drugs and their breakdown products in the urine can interfere with the analysis. They should be discontinued whenever possible, or the laboratory should be informed (salicylates, tetracyclines, sympathomimetic and anti-hypertensive drugs).

α) Vanilmandelic Acid

40% of the catecholamines (epinephrine and norepinephrine) are excreted as 3-methoxy-4-hydroxymandelic acid (VMA). PISANO'S method has proved to be the most suitable and is very reliable for routine examination. It involves extraction, oxidation and absorption measurement.

Interpretation: Healthy adults excrete between 1 and 7 mg vanilmandelic acid in 24 hours. In children these values are lower according to age.

The excretion is usually increased in cases of pheochromocytoma. Elevated values can also be detected in cases suffering from tumors of the sympathetic nerve tissue in which clinical symptoms due to overproduction of catecholamines are absent.

β) Metanephrine and Normetanephrine

Less than 1 mg of these metabolites of epinephrine and norepinephrine is normally excreted in a 24-hour urine sample. In exceptional cases, demonstration of these breakdown products is more reliable for diagnostic purposes than the estimation of VMA.

γ) Epinephrine, Norepinephrine, and Dopamine

Only 3–7% of epinephrine and norepinephrine are excreted unchanged in the urine. As the amounts lie in the μg range they can be estimated only by fluorometric methods.

Normal values: 10–30 μg of epinephrine in 24 hours, 20–60 μg of norepinephrine in 24 hours. Rule of thumb for normal values: free epinephrine plus norepinephrine below 100 μg in 24 hours. Increased values of dopamine, the precursor of epinephrine and norepinephrine, can indicate malignancy. This investigation is of particular importance in pediatrics for the detection of immature tumors of the sympathetic nerve tissue.

c) Localization of the Tumor

α) Catecholamine Excretion

A preponderance of epinephrine production suggests a tumor situated in the adrenal medulla. Excessive norepinephrine production is due to an extra-adrenal tumor in one third of cases.

β) Radiological Investigations

A chest X-ray and an intravenous pyelogram are routinely performed when a pheochromocytoma is suspected. On the other hand, aortography or a retropneumoperitoneum with tomography may involve serious risks of hypertensive crises and hemorrhage into the tumor with subsequent shock.

These investigations allow localization of the tumor in 60% of cases, but they demand meticulous supervision of the patient and immediate treatment of any complicating incidents. We perform venous catherization rather than retropneumoperitoneum and aortography. Brown fat ("Hibernoma") an immature form of adipose tissue, devellops occasionally in proximity to pheochromocytomas and may mimick a tumor by its extensive and irregular vascularisation (LEIPHART, 1970; FEYRTER, 1971).

γ) Catheterization of the Vena Cava

The catheter is introduced into the femoral vein and an X-ray monitor is used in guiding it along the inferior vena cava. Samples of blood are taken at different levels for the estimation of catecholamines. The highest values are found selectively in the vein draining the tumor or in the region of the venous inflow from the tumor. This method allows determination both of the level at which the tumor is situated and of which side is affected, which makes selection of the optimal surgical approach considerably easier (see Chap. VII, p. 352).

This procedure has proved not to be too strenuous for patients, but requires a special laboratory.

Estimation of catecholamines in the plasma by spectrofluorometric methods is very ambitious and detects plasma concentrations of 0.05 μg/l in the case of epinephrine, and of 0.45 μg/l in that of norepinephrine.

Biological methods of estimation have now been superseded by biochemical methods for clinical purposes.

6. Course and Prognosis

The prognosis depends on early recognition and treatment of the pheochromocytoma. Left untreated, the condition can continue for years and death can occur at any time due to apoplexy, pulmonary edema, myocardial infarction, or sequels of malignant hypertension. Spontaneous healing due to infarction of the tumor is possible but extremely rare. Patients are in constant danger of fatal shock induced by situations such as excitement, minor trauma, and surgery, which cause nervous stimulation of the adrenal medulla. As discussed above, attacks usually increase in frequency and intensity. No general life expectancy can be given. Successful surgery can bring about complete cure as long as the kidneys and circulatory organs are not irreversibly damaged. Pregnancy can lead to manifestation of the disorder. Immediate surgery is indicated since mother and child are both in danger (abortion).

7. Differential Diagnosis

Hypertension is the first condition to be considered in the differential diagnosis of the pheochromocytoma with persistent hypertension. Every hypertensive patient below the age of 40 should be investigated for a pheochromocytoma. Loss of weight and tachycardia can simulate hyperthyroidism. Lead poisoning with pallor, hypertension, and abdominal colic has certain similarities to the pheochromocytoma.

In the differential diagnosis of the paroxysmal form, the following conditions must be considered: headaches associated with encephalopathy, vasomotor crises, migraine (since nausea, headaches and scintillating scotoma can also arise with a pheochromocytoma), arteriosclerotic cerebral circulatory disturbances, hyperinsulinism, and carcinoid syndrome. The term "pseudopheochromocytoma" should be avoided for description of tumors in the region of the kidneys which are sometimes associated with blood pressure variations due to phentolamine and histamine but no evidence of increased catecholamine formation.

On the other hand, pheochromocytomas can be combined with renal hypertension due to renal ischemia caused by mechanical effects of the tumor (WEIDMANN, 1968).

Autonomous hyperreflexia in paraplegics with a transverse lesion of the spinal cord above thoracal 7 can lead to over-distension of the bladder, which in turn gives rise to the release of large amounts of norepinephrine accompanied by corresponding symptoms (GARNIER, 1964).

8. Treatment

Palliative medical treatment is an essential part of the presurgical preparation of patients. It is also indicated if the general condition of the patient does not premit surgical intervention and in cases of inoperable malignant pheochromocytoma. The α-receptor blocker phenoxybenzamine (Dibenyline) has proved suitable. It is given orally in high dosages ranging from 5 to 60 mg per day. The dose should be divided over the day, and the optimal dose must be determined individually. Hypovolemia is nearly always present and abates after 5–7 days of treatment, as is indicated by the fall in hemoglobin. Copious blood transfusions complete the preparation for surgery. This treatment improves the persistent hypertension at the same time and prevents hypertensive attacks.

Patients undergoing long-term treatment with high doses of dibenyline often suffer from orthostatic hypotension which can itself cause them to become completely bedridden.

α-Methyl-dopa (Aldomet), an antihypertensive drug, has not proved suitable for this treatment. α-Methyl-para-tyrosine promises to be effective although clinical experience is limited.

Guanethidine and ganglionic blocking agents are contraindicated in the treatment of pheochromocytomas since the sensitivity of end organs to norepinephrine rises under their influence.

Treatment must be aimed at surgical removal of the pheochromocytoma, which can bring about a complete cure.

If the diagnosis is confirmed and the tumor has been localized by radiography or venous catheterization, there is not great technical difficulty attached to the operation. Close teamwork is essential between surgeon, internist and anesthesiologist, who must all be very familiar with surgery of pheochromocytoma.

Pretreatment with phenoxybenzamine is now obligatory, since preoperative correction of the hypovolemia can prevent dangerous states of collapse immediately after surgical removal of the tumor. Propranolol (Inderal), a β-receptor blocking agent, should only be used (dosage: 15–45 mg/day) if there is tachycardia of more than 140.

Any narcotic agent, and particularly curare, can induce a blood pressure crisis even when ganglionic blockers have been avoided. Phentolamine must be kept ready for intravenous application in case of emergency. Continuous monitoring of blood pressure and ECG is advisable. A certain amount of risk due to anesthesia cannot be overcome in older patients, and anesthesia should only be used for the operation and not for diagnostic procedures such as aortography.

Although pheochromocytomas occur bilaterally in about one tenth of cases, so that revision of both sides is desirable, the less drastic dorsolumbar approach can be chosen for surgery if the tumor has been localized with certainty; if the tumor is found, exploration of the other side is avoided. Other authors prefer the transabdominal route via a transverse or longitudinal incision which permits inspection of both adrenals and their surroundings.

Cortisol hemisuccinate must be at hand in cases of bilateral pheochromocytomas where bilateral total adrenalectomy is essential. Doses (see p. 330) are the same as for adrenalectomy.

When the pheochromocytoma is located, it should be palpated and squeezed as little as possible to minimize the risk of inducing a hypertensive attack. The blood supply must be ligated as quickly as possible and the tumor removed intact. The whole of the adrenal involved must be removed because of the risk of recurrences. This principle can be disregarded in cases where the pheochromocytoma is topographically completely separated from the adrenal gland. Only in cases of bilateral pheochromocytomas should functioning adrenal tissue be left behind where possible.

Surgical treatment of pheochromocytoma is extremely rewarding, except in cases of malignancy or permanent hypertension. Patients can be completely cured. If the blood pressure does not fall immediately after the operation, it is possible that a second active tumor is present. This sign, however, is no longer entirely reliable because of the premedication with phenoxybenzamine. These cases must be explored with particular care. On the other hand, it has been known for hypertension to persist after removal of the tumor and the blood pressure to return to normal values only after some weeks or months. Hypertension can, however, become permanent in cases of long duration.

References

Embryology and Anatomy

Arnold, M., Hager, G.: Funktionsentwicklung der Nebenniere beim Goldhamster: Elektronenmikroskopische Untersuchungen am Mark. Z. Zellforsch. **83**, 117 (1967).

Elfvin, L.G.: The ultrastructure of the capillary fenestrae in the adrenal medulla of the rat. J. Ultrastruct. Res. **12**, 687 (1965).

— The development of the secretory granules in the rat adrenal medulla. J. Ultrastruct. Res. **17**, 45 (1967).

— Effects of reserpine on the surface structure of chromaffin cells in the rat adrenal medulla. J. Ultrastruct. Res. **21**, 459 (1968).

— Appelgren, L.E., Ullberg, S.: High resolution autoradiography of the adrenal medulla after injection of tritiated dihydroxyphenylalanine (DOPA). J. Ultrastruct. Res. **14**, 277 (1966).

Hempel, K., Kraft, M.: Zum Brenzkatechinamin-Stoffwechsel der Nebenniere. Licht- und elektronenmikroskopisch-autoradiographische Untersuchungen mit ^{3}H-Dopa an Mäusen, Z. Zellforsch. **85**, 322 (1968).

Moppert, J.: Zur Ultrastruktur der phaeochromen Zellen im Nebennierenmark der Ratte. Z. Zellforsch. **74**, 32 (1966a).

— Der intrazelluläre Bildungsort der phaeochromen Granula im Nebennierenmark der Ratte. Z. Zellforsch. **74**, 45 (1966b).

Stöhr, Jr., P.H.: Lehrbuch der Histologie und der mikroskopischen Anatomie. Berlin-Göttingen-Heidelberg: Springer 1951.

Unsicker, K.: Über die Ganglienzellen im Nebennierenmark des Goldhamsters. Ein Beitrag zur Frage der peripheren Neurosekretion. Z. Zellforsch. **76**, 187 (1967).

Biochemistry, Physiology and Pharmacology of the Catecholamines

AXELROD, J.: The metabolism, storage and release of catecholamines. Recent Progr. Hormone Res. **21**, 597 (1965).

BALTZAN, M.A., ANDRES, R., CADER, G., ZIERLER, K.L.: Effects of epinephrine on forearm blood flow and metabolism in man. J. clin. Invest. **44**, 80 (1965).

BELLEAU, B.: Steric effects in catecholamine interactions with enzymes and receptors. Pharmacol. Rev. **18**, 131 (1966).

BLASCHKO, H., MUSCHOLL, E. (ed.): Catecholamines. Handbuch der experimentellen Pharmakologie, vol. 33. Berlin-Heidelberg-New York: Springer 1972.

BRAUNWALD, E., HARRISON, D.C., CHIDSEY, C.A.: The heart as an endocrine organ. Amer. J. Med. **36**, 1 (1964).

BURN, J.H.: Release of noradrenaline from the sympathetic postganglionic fibre. Brit. med. J. **1967I**, 197.

EPSTEIN, ST.E., BRAUNWALD, E.: Beta-adrenergic receptor blocking drugs. Mechanisms of action and clinical applications. New Engl. J. Med. **275**, 1106 (1966).

HARRISON, T.S., CHAWLA, R.C., WOJTALIK, R.S.: Steroidal influences on catecholamines. New Engl. J. Med. **279**, 136 (1968).

HENKIN, R.I., BARTTER, F.C.: Urinary free and conjugated epinephrine and norepinephrine in normal subjects and in patients with various disorders. Fed. Proc. **24**, 133 (1965).

HESS, W.R.: Die funktionelle Organisation des vegetativen Nervensystems. Basel: Benno Schwabe 1948.

HOLTZ, P.: Gewebs- und Neurohormone. 8. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1962.

— PALM, D.: Brenzkatechinamine und andere sympathicomimetische Amine. In: Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie, Bd. 58. Berlin-Heidelberg-New York: Springer 1966.

ILLIG, R., ZIEGLER, W.H.: Glucose tolerance and immunoreactive insulin in patients with pheochromocytoma: the effect of α-receptor blocking agents. Acta endocr. (Kbh.) **66**, 368 (1971).

IVERSEN, L.L. (ed.): Catecholamines. Brit. med. Bull. **29**, 91 (1973).

KING, S.E., BALDWIN, D.S.: Production of renal ischemia and proteinuria in men by the adrenal medullary hormones. Amer. J. Med. **20**, 217 (1956).

KUSCHINSKY, G., SÜLLMANN, H.: Kurzes Lehrbuch der Pharmakologie. Berlin-Göttingen-Heidelberg: Springer 1964.

LABHART, A., SCHEITLIN, W.: Niere bei Endokrinopathien. In: Handbuch der inneren Medizin, hrsg. von SCHWIEGK, Bd. 8, 3. Teil, 5. Aufl. Berlin-Heidelberg-New York: Springer 1968.

LANGEMANN, H.: Pharmakologie des vegetativen Nervensystems. Akt. Fragen Psychiat. Neurol. **3**, 74 (1966).

MACCANNELL, K.L., MCNAY, J.L., MEYER, M.B., GOLDBERG, L.I.: Dopamine in the treatment of hypotension and shock. New Engl. J. Med. **275**, 1389 (1966).

MALMEJAC, J.: Activity of the adrenal medulla and its regulation. Phys. Rev. **44**, 186 (1964).

MCDONALD, R.H., JR., GOLDBERG, L.I., MCNAY, J.L., TUTTLE, E.P.: Effect of dopamine in man: augmentation of sodium excretion, glomerular filtration rate, and renal plasma flow. J. clin. Invest. **43**, 1116 (1964).

MUELLER, C.B., SURTSCHIN, A., CARLIN, M.R., WHITE, H.L.: Glomerular and tubular influences on sodium and water excretion. Amer. J. Physiol. **165**, 411 (1951).

NICKEL, J.F., MCSMYTHE, CH., PAPPER, E.M., BRADLEY, S.E.: A study of the mode of action of the adrenal medullary hormones on sodium, potassium and water excretion in man. J. clin. Invest. **33**, 1687 (1954).

PULLMAN, T.N., MCCLURE, W.W.: The effect of l-noradrenaline on electrolyte excretion in normal man. J. Lab. clin. Med. **39**, 711 (1952).

READER, R.: Catecholamines in cardiovascular physiology and disease. New York: Amer. Heart Assoc., Inc. 1967.

SANDLER, M.: Biosynthesis and metabolism of the catecholamines. Schweiz. med. Wschr. **100**, 526 (1970).

SCHÜRMANN, H.J.: Speicherung und Freisetzung der Brenzcatechinamine. Gewebs- u. Neurohormone. 8. Symp. der Dtsch. Ges. Endocrin. Berlin-Göttingen-Heidelberg: Springer 1962.

Second Symposium on Catecholamines 1965. Pharmacol. Rev. **18**, 1—804 (1966).

SUTHERLAND, E.W., ØYE, I., BUTCHER, R.W.: The action of epinephrine and the role of the adenyl cyclase system in hormone action. Recent Progr. Hormone Res. **21**, 623 (1965).

— ROBINSON, A., BUTCHER, R.W.: Some aspects of the biological role of adenosine 3′,5′-monophosphate (cyclic AMP). Circulation **37**, 279 (1968).

SUNDIN, T.: The effect of body posture on the urinary excretion of adrenaline and noradrenaline. Acta med. scand., Suppl. **336** (1958).

THOENEN, H.: Bildung und funktionelle Bedeutung adrenerger Ersatztransmitter. Berlin-Heidelberg-New York: Springer 1969.

VANCE, J.E., BUCHANAN, R.D., O'HARA, D., WILLIAMS, R.H., PORTE, D., JR.: Insulin and glucagon responses in subjects with pheochromocytoma: effect of alpha adrenergic blockade. J. clin. Endocr. **29**, 911 (1969).

VOGT, M.: Effects of drugs on metabolism of catecholamines in the brain. Brit. med. Bull. **21**, 57 (1965).

WEINER, N.: The catecholamines: biosynthesis, storage and release, metabolism and metabolic effects. In: The hormones, ed. by PINCUS, THIMANN and ASTWOOD, vol. IV. New York and London: Academic Press 1964.

WESSON, L.G.: Hormonal influences on renal function. Ann. Rev. Med. **12**, 77 (1961).

WILBER, J.F., TURTLE, J.R., CRANE, N.A.: Inhibition of insulin secretion by a phaeochromocytoma. Lancet **1966II**, 733.

WURTMAN, R.J.: Catecholamines. New Engl. J. Med. **273**, 637, 693, 746 (1965).

— AXELROD, J.: Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. J. biol. Chem. **241**, 2301 (1966).

— POHORECKY, L.A.: Adrenocortical control of epinephrine synthesis in health and disease. Advanc. metabol. Disorders **5**, 53 (1971).

Adrenal Medulla: Hypofunction

BROBERGER, O., ZETTERSTRÖM, R.: Hypoglycemia with an inability to increase the epinephrine secretion in insulin-induced hypoglycemia. J. Pediat. **59**, 215 (1961).

BRUNJES, SH., HODGMAN, J., NOWACK, J., JOHNS, V.J.: Adrenal medullary function in idiopathic spontaneous hypoglycemia of infancy and childhood. Amer. J. Med. **34**, 168 (1963).

DANCIS, J., SMITH, A.A.: Familial dysautonomia. New Engl. J. Med. **274**, 207 (1966).

EULER, U.S. v., IKKOS, D., LUFT, R.: Adrenaline excretion during resting conditions and after insulin in adrenalectomized human subjects. Acta endocr. (Kbh.) **38**, 441 (1961).

GOLDFIEN, A., MOORE, R., ZILELI, S., HAVENS, L.L., BOLING, L., THORN, G.W.: Plasma epinephrine and norepinephrine levels during insulin-induced hypoglycemia in man. J. clin. Endocr. **21**, 196 (1961).

GOODALL, M.C., GITLOW, S.E., ALTON, H.: Decreased noradrenaline (norepinephrine) synthesis in familial dysautonomia. J. clin. Invest. **50**, 2734 (1971).

— HAYNES, B.W.: Adrenal medullary insufficiency in severe thermal burn. J. clin. Invest. **39**, 1927 (1960).

HICKLER, R.B., WELLS, R.E., TYLER, H.R., HAMLIN, J.T.: Plasma catecholamine and electroencephalographic responses to acute postural change. Amer. J. Med. **26**, 410 (1959).
LUFT, R., EULER, U.S.v.: Two cases of postural hypotension showing a deficiency in release of nor-epinephrine and epinephrine. J. clin. Invest. **32**, 1065 (1953).
MARK, G.: Die idiopathische orthostatische Hypotonie. Schweiz. med. Wschr. **99**, 1877 (1969).
SLATON, P.E., JR., BIGLIERI, E.G.: Reduced aldosterone excretion in patients with autonomic insufficiency. J. clin. Endocr. **27**, 37 (1967).
SMITH, A.A., TAYLOR, T., WORTIS, S.B.: Abnormal catecholamine metabolism in familial dysautonomia. New Engl. J. Med. **268**, 705 (1963).
TIETZE, H.U., ZURBRÜGG, R.P., ZUPPINGER, K.A., JOSS, E.E., KÄSER, H.: Occurrence of impaired cortisol regulation in children with hypoglycemia associated with adrenal medullary hyporesponsiveness. J. clin. Endocr. **34**, 948 (1972).
— — — KÄSER, H.: Die Kortisolregulation bei kindlichen Hypoglykämien mit Nebennierenmark-Dysregulation. Schweiz. med. Wschr. **100**, 545 (1970).
VÖLLM, K., SCHAUB, F.: Zur sogenannten „Postural Hypotension". Lageabhängige Hypotonie bei idiopathischer Störung der neurovegetativen Kreislaufregulation. Schweiz. med. Wschr. **94**, 33 (1964).
WEINSHILBOUM, R.M., AXELROD, J.: Reduced plasma dopamine-β-hydroxylase activity in familial dysautonomia. New Engl. J. Med. **285**, 938 (1971).

Pheochromocytoma

Reviews

CROUT, J.R.: Pheochromocytoma. Pharmacol. Rev. **18**, 651 (1966).
GREER, W.E.R., ROBERTSON, CH.W., SMITHWICK, R.H.: Pheochromocytoma. Diagnosis, operative experiences and clinical results. Amer. J. Surg. **107**, 192 (1964).
HERMANN, H., MORNEX, R.: Les phéochromocytomes. Etude clinique et physiopathologique des tumeurs humaines secrétants des catécholamines. Paris: Gauthier-Villars 1964.
HILLESTAD, L., BRODWALL, E.: Pheochromocytoma. A review of clinical findings in 10 cases. Acta med. scand. **187**, 313 (1970).
HUME, D.M.: Phaeochromocytomas in the adult and in the child. Amer. J. Surg. **99**, 458 (1960).
— Phaeochromocytoma. In: Clinical endocrinology, vol. II, ed. by ASTWOOD and CASSIDY. New York/London: Grune & Stratton 1968.
SACK, H., KOLL, J.F.: Das Phäochromocytom. Ergebn. inn. Med. Kinderheilk. **19**, 446 (1963).
SCHWARZ, K.: Das Phäochromocytom. Internist (Berl.) **5**, 22 (1964).
ZIEGLER, W.H., LANGEMANN, H., MÜLLER, P.B.: Vergleichende Untersuchungen an Tumorgewebe von Phaeochromocytom und Carcinoid. Schweiz. med. Wschr. **97**, 1731 (1967).

Pathological Anatomy

BRINES, O.A., JENNINGS, E.R.: Paragangliomas. Review of subject and report of 5 original cases. Amer. J. Path. **24**, 1167 (1948).
DIETRICH, A., SIEGMUND, H.: Die Nebennieren und das chromaffine System (Paraganglien, Carotisdrüse, Steißdrüse). In: F. HENKE u. O. LUBARSCH, Handbuch der speziellen pathologischen Anatomie, Bd. 8, p. 951. Berlin: Springer 1926.
GRAHAM, J.B.: Pheochromocytoma and hypertension: An analysis of 207 cases. Int. Abstr. Surg. **92**, 105 (1951).
KARSNER, H.T.: Tumors of the adrenal. In: Atlas of tumor pathology, Sect. VIII, Fasc. 29. Washington, D.C.: Armed Forces Inst. of Path. 1950.
LIEBEGOTT, G.: Studien zur Orthologie und Pathologie der Nebennieren. Beitr. path. Anat. **109**, 93 (1947).
— Die Pathologie der Nebennieren. Verh. dtsch. path. Ges. **36**, 21 (1952).
SYMINGTON, TH.: Functional pathology of the human adrenal gland. Edinburgh-London: Livingston Ltd. 1969.
ZOLLINGER, H.U.: Nierenveränderungen bei Phäochromocytom. Schweiz. med. Wschr. **89**, 841 (1959).

Clinical Aspects and Therapy of the Paroxysmal and Persisting Hypertension

BAIRD, J.M., COHEN, H.: Pheochromocytoma: A case with hypotension, paroxysmal hypertension and urinary retention. Lancet **1954II**, 270.
BELLAS, J.E.: Nonsurgical pheochromocytomas. J. Amer. med. Ass. **185**, 601 (1963).
BRUNJES, S., JOHNS, V.J., JR., CRANE, M.G.: Phaeochromocytoma. Postoperative shock and blood volume. New Engl. J. Med. **262**, 393 (1960).
BUCHBORN, E.: Klinische Pathophysiologie der Harnkonzentrierung. Schweiz. med. Wschr. **94**, 1273 (1964).
CAHILL, G.F.: Pheochromocytomas. J. Amer. med. Ass. **138**, 180 (1948).
CRANDELL, D., L., MYERS, R.T.: Phaeochromocytoma. Anesthetic and surgical considerations. J. Amer. med. Ass. **187**, 12 (1964).
CROUT, J.R., SJOERDSMA, A.: Turnover and metabolism of catecholamines in patients with pheochromocytoma. J. clin. Invest. **43**, 94 (1964).
DAHL-IVERSEN, E.: 18 Phaeochromocytomas. Clinical aspects and surgical results. Acta chir. scand. **116**, 118 (1959).
DRUKKER, W., FORMEIJNE, P., SCHOOT, J.B. VAN DER: Hyperplasia of the adrenal medulla. Brit. med. J. **1957I**, 186.
ENGELMAN, K., HORWITZ, D., JÉQUIER, E., SJOERDSMA, A.: Biochemical and pharmacologic effects of α-methyltyrosine in man. J. clin. Invest. **47**, 577 (1968).
— MUELLER, P.S., SJOERDSMA, A.: Elevated plasma free fatty acid concentrations in patients with pheochromocytoma. New Engl. J. Med. **270**, 865 (1964).
— SJOERDSMA, A.: Chronic medical therapy for pheochromocytoma. Ann. intern. Med. **61**, 229 (1964).
FERTIG, H.A., TAYLOR, R.D., CORCORAN, A.C., PAGE, I.H.: The renal manifestations of phaeochromocytoma. Report of a case. Ann. intern. Med. **35**, 1358 (1951).
GIFFORD, R.W., JR., KVALE, W.F., MAHER, F.T., ROTH, G.M., PRIESTLEY, J.T.: Clinical features, diagnosis and treatment of pheochromocytoma: a review of 76 cases. Proc. Mayo Clin. **39**, 281 (1964).
GRAHAM, J.B.: Pheochromocytoma and hypertension. An analysis of 207 cases. Int. Abstr. Surg. **92**, 105 (1951).
GREEN, D.M.: Phaeochromocytoma and chronic hypertension. J. Amer. med. Ass. **131**, 1260 (1946).
HARRISON, J.H., GARDNER, F.H., DAMMIN, G.J.: A note on pheochromocytoma and renal hypertension. J. Urol. (Baltimore) **79**, 173 (1958).
KOLL, J.F., SACK, H.: Besonderheiten des Kreislaufverhaltens bei Phaeochromocytomen mit Dauerhochdruck. Münch. med. Wschr. **104**, 655 (1962).
KVALE, W.F., ROTH, G.M., MANGER, W.M., PRIESTLEY, J.T.: Pheochromocytoma. Circulation **14**, 622 (1956).
LABHART, A., SCHEITLIN, W.: Niere bei Endokrinopathien. In: Handbuch der inneren Medizin, ed. by SCHWIEGK, Bd. 8, 3. Teil, 5th ed. Berlin-Heidelberg-New York: Springer 1968.

MATHISON, D.A., WATERHOUSE, C.A.: Cushing's syndrome with hypertensive crisis and mixed adrenal cortical adenoma-pheochromocytoma (corticomedullary adenoma). Amer. J. Med. **47**, 635 (1969).

MEGEVAND, R., SCHNEITER, R.: Un cas de phéochromocytome extrasurrénalien droit à symptomatologie essentiellement digestive et douloureuse. Schweiz. med. Wschr. **13**, 498 (1963).

MONTALBANO, F.P., BARONOFSKY, I.D., BALL, H.: Hyperplasia of the adrenal medulla. A clinical entity. J. Amer. med. Ass. **182**, 164 (1962).

ROSENHEIM, M.L., ROSS, E.J., WRONG, O.M., HODSON, C.J., DAVIES, D.R., SMITH, J.F.: Unilateral renal ischemia due to compression of a renal artery by a pheochromocytoma. Amer. J. Med. **34**, 735 (1963).

SACK, H., KOLL, J.F.: Das Phäochromocytom. Ergebn. inn. Med. Kinderheilk. **19**, 446 (1963).

SJOERDSMA, A., ENGELMAN, K., SPECTOR, S., UDENFRIEND, S.: Inhibition of catecholamine synthesis in man with alpha-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. Lancet **1965II**, 1092.

SMITHWICK, R.H., GREER, W.E., ROBERTSON, C.W., WILKINS, R.W.: Phaeochromocytoma. New Engl. J. Med. **242**, 252 (1950).

SPERGEL, G., BLEICHER, S.J., ERTEL, N.H.: Carbohydrate and fat metabolism in patients with pheochromocytoma. New Engl. J. Med. **278**, 803 (1968).

THOMAS, J.E., ROOKE, D., KVALE, W.F.: The neurologists experience with pheochromocytoma. J. Amer. med. Ass. **197**, 754 (1966).

VLIET, P.D. VAN, BURCHELL, H.B., TITUS, J.L.: Focal myocarditis with pheochromocytoma. New Engl. J. Med. **274**, 1102 (1966).

WEIDMANN, P., SIEGENTHALER, W., ZIEGLER, W.H., SULSER, H., ENDRES, P., WERNING, C.: Hypertension associated with tumors adjacent to renal arteries. Amer. J. Med. **47**, 528 (1969).

WILBER, J.F., TURTLE, J.R., CRANE, N.A.: Inhibition of insulin secretion by a phaeochromocytoma. Lancet **1966II**, 733.

ZIEGLER, W., LABHART, A., FRICK, P.: Schockverhütung beim Phäochromocytom durch präoperative Behandlung mit einem α-Rezeptorenblocker. Bibl. cardiol. (Basel) **17**, 1 (1966).

ZOLLINGER, H.U.: Nierenveränderungen bei Phäochromocytom. Schweiz. med. Wschr. **89**, 841 (1959).

Hypotension

KAHN, M.T., MULLON, D.A.: Pheochromocytoma without hypertension. J. Amer. med. Ass. **188**, 74 (1964).

LOIZEAU, E.: A propos d'un cas de phéochromocytome avec hypotension artérielle. Schweiz. med. Wschr. **94**, 926 (1964).

LOUIS, W.J., DOYLE, A.E., HEARTH, W.C., ROBINSON, M.J.: Secretion of Dopa in phalochromocytoma. Brit. med. J. **4**, 325 (1972).

PAGE, L.B., RAKER, J.W., BERBERICH, F.R.: Pheochromocytoma with predominant epinephrine secretion. Amer. J. Med. **47**, 648 (1969).

RICHMOND, J., FRAZER, S.C., MILLAR, D.R.: Paroxysmal hypotension due to an adrenaline-secreting phaeochromocytoma. Lancet **1961II**, 904.

Malignant Pheochromocytomas

FORSELL, J., MALM, P.: Recurrent pheochromocytoma. Acta med. scand. **163**, 55 (1959).

KÄSER, H.: Catecholamine-producing neural tumors other than pheochromocytoma. Pharmacol. Rev. **18**, 659 (1966).

— TÜRLER, K., BURRI, M.C.: Zum Katecholaminstoffwechsel im Gewebe maligner und benigner Tumoren des sympathischen Nervensystems. Schweiz. med. Wschr. **101**, 484 (1971).

KARLSON, P., SEKERIS, C.E., HERRLICH, P.: Über neue Dopaminmetaboliten im Harn beim bösartigen Phäochromocytom. Dtsch. med. Wschr. **39**, 1873 (1963).

PALMIERI, G., IKKOS, D., LUFT, R.: Malignant phaeochromocytoma. Acta endocr. (Kbh.) **36**, 549 (1961).

SHEPS, S.G., KOTTKE, B.A., TYCE, G.M., FLOCK, E.V.: Effect of methyldopa on the metabolism of catecholamines and tryptophan in metastatic pheochromocytoma. Amer. J. Cardiol. **14**, 641 (1964).

Special Forms and Combinations with Other Syndromes

CUNLIFFE, W.J., BLACK, M.N., HALL, R., JOHNSTON, J.D.A., HUDGSON, P., SHUSTER, S., GUDMUNSSON, P.V., JOPLIN, G.F., WILLIAMS, E.D., WOODHOUSE, N.J.Y., GALANTE, L., MACINTYRE, J.: A calcitonin-secreting thyroid carcinoma. Lancet **1968II**, 63.

CUSHMAN, P., JR.: Familial endocrine tumors. Report of two unrelated kindred affected with pheochromocytomas, one also with multiple thyroid carcinomas. Amer. J. Med. **32**, 352 (1962).

DUKE, W.M., PHILLIPS, M.W., DONALS, J.M., BOSHELL, B.R.: A norepinephrine-secreting glomic tissue tumor (Chemodectoma). J. Amer. med. Ass. **193**, 20 (1965).

GARNIER, B., GERTSCH, R.: Autonome Hyperreflexie und Katecholaminausscheidung beim Paraplegiker. Schweiz. med. Wschr. **94**, 124 (1964).

GLUSHIEN, A.S., MANSUY, M.M., LITTMAN, D.S.: Pheochromocytoma. Its relationship to the neurocutaneous syndromes. Amer. J. Med. **14**, 318 (1953).

HUANG, S., MCLEISH, W.A.: Pheochromocytoma and medullary carcinoma of thyroid. Cancer (Philad.) **21**, 302 (1968).

KEYNES, W.M., TILL, A.S.: Medullary carcinoma of the thyroid gland. Quart. J. Med., N.S. **40**, 443 (1971).

Leading article: Phaeochromocytoma and thyroid cancer. Brit. med. J. **1965II**, 549.

LEVIT, S.A., SHEPS, S.G., ESPINOSA, R.E., REMINE, W.H., HARRISON, E.G., JR.: Catecholamine-secreting paraganglioma of glomus-jugulare region resembling pheochromocytoma. New Engl. J. Med. **281**, 805 (1969).

LJUNGBERG, O., CEDERQUIST, E., STUDNITZ, W. v.: Medullary thyroid carcinoma and phaeochromocytoma: a familial chromaffinomatosis. Brit. med. J. **1967I**, 279.

MATHYS, S., ZIEGLER, W.H., FRANCKE, C.: Bilaterales Phäochromozytom — medulläres Schilddrüsenkarzinom mit Cushing-Syndrom. Schweiz. med. Wschr. **102**, 798 (1972).

MELVIN, K.E.B., TASHJIAN, A.H., JR.: The syndrome of excessive thyrocalcitonin produced by medullary carcinoma of the thyroid. Proc. nat. Acad. Sci. (Wash.) **59**, 1216 (1968).

NAGANT DE DEUXCHAISNES, C., FANCONI, A., ALBERTO, P., RUDLER, J.C., MACH, R.S.: Phéochromocytomes extrasurrénaliens multiples avec «dystrophie d'Albright» et hémiangiomes cutanés. Schweiz. med. Wschr. **90**, 886 (1960).

ROSSIER, A.B., ZIEGLER, W.H., DUCHOSAL, P.W., MEYLAN, J.: Fonction sexuelle, dysréflexie autonome et catécholamines dans les lésions médullaires traumatiques. Schweiz. med. Wschr. **101**, 784 (1971).

SAPIRA, J.S., ALTMAN, M., VANDYK, K., SHAPIRO, A.P.: Bilateral adrenal pheochromocytoma and medullary thyroid carcinoma. New Engl. J. Med. **273**, 140 (1965).

SCHIMKE, R.N., HARTMANN, W.H., PROUT, T.E., RIMOIN, D.L.: Pheochromocytoma, medullary thyroid carcinoma and multiple neuromas. New Engl. J. Med. **279**, 1 (1968).

SCHLEGEL, G.G.: Neurofibromatose Recklinghausen und Phäochromocytom. Schweiz. med. Wschr. **90**, 31 (1960).

STEINER, H. L., GOODMAN, A. D., POWERS, S. R.: Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrinopathia type 2. Medicine (Baltimore) **47**, 371 (1968).
STREIT, W., BAUMANN, R. P.: Phäochromocytom-Ganglioneurom-Fibrosarkom. Schweiz. med. Wschr. **97**, 1152 (1967).
TISHERMAN, S. E., GREGG, F. J., DANOWSKI, T. S.: Familial phaeochromocytoma. J. Amer. med. Ass. **182**, 152 (1962).
WILLIAMS, E. D.: Histogenesis of medullary carcinoma of the thyroid. J. clin. Path. **19**, 114 (1966).
— POLLOCK, D. J.: Multiple mucosal neuromata with endocrine tumours: a syndrome allied to von Recklinghausen's disease. J. Path. Bact. **91**, 71 (1966).

Diagnosis

ENGELMAN, K., SJOERDSMA, A.: A new test for phaeochromocytoma. Pressor responsiveness to Tyramine. J. Amer. med. Ass. **189**, 81 (1964).
FEYRTER, F.: Ein adrenolipoides Syndrom: Normologie und Pathologie des braunen Fettgewebes. Wien. klin. Wschr. **83**, 393 (1971).
GITLOW, S. E., MENDLOWITZ, M., KHASSIS, S., COHEN, G., STRA, J.: The diagnosis of pheochromocytoma by determination of urinary 3-methoxy, 4-hydroxymandelic acid. J. clin. Invest. **39**, 211 (1960).
JÉQUIER, E.: Mesure biochimique de la fonction de la médullosurrénale et du système sympathique en clinique. Schweiz. med. Wschr. **100**, 532 (1970).
KÄSER, H.: Die Bedeutung der 3-Methoxy-4-Hydroxy-Mandelsäure (MHMS) für die Differentialdiagnostik neuraler Tumoren im Kindesalter. Schweiz. med. Wschr. **91**, 586 (1961).
— Biochemische Diagnostik des Phäochromocytoms, des Neuroblastoms und anderer neuro-ektodermaler Neoplasien. Helv. paediat. Acta, Suppl. 29 (1972).
— Catecholamine-producing neural tumors other than pheochromocytoma. Pharmacol. Rev. **18**, 659 (1966).
LAWRENCE, A. M.: Glucagon provocative test for pheochromocytoma. Ann. intern. Med. **66**, 1091 (1967).
LEFEBVRE, P. J., CESSION-FOSSION, A., LUYCKX, A. S.: Glucagon test for pheochromocytoma. Lancet **1966II**, 1366.
LEIPHART, CH. J., NUDELMAN, E. J.: Hibernoma masquerading as a pheochromocytoma: case report. Radiology **95**, 659 (1970).
PISANO, J. J., CROUT, J. R., ABRAHAM, D.: Determination of 3-methoxy-4-hydroxymandelic acid in urine. Clin. chim. Acta **7**, 285 (1962).
ROSSI, P., YOUNG, J. S., PANKE, W. F.: Techniques, usefullness and hazards of arteriography of pheochromocytoma. J. Amer. med. Ass. **205**, 547 (1968).
ROTH, G. M., FLOCK, E. V., KVALE, W. F., WAUGH, J. M., OGG, J.: Pharmacologic and chemical test as an aid in the diagnosis of pheochromocytoma. Circulation **21**, 769 (1960).
SATO, T. L., SJOERDSMA, A.: Urinary homovanillic acid in phaeochromocytoma. Brit. med. J. **1965II**, 1472.
SHEPS, SH. G., MAHER, F. T.: Comparison of the histamine and tyramine tests in the diagnosis of pheochromocytoma. J. Amer. med. Ass. **195**, 265 (1966).
— — Histamin and glucagon tests in diagnosis of pheochromocytoma. J. Amer. med. Ass. **205**, 895 (1968).
SPENGLER, G. A., KÄSER, H., RIVA, G.: Die Bestimmung von Metanephrin und Normetanephrin im Urin. Schweiz. med. Wschr. **93**, 1684 (1963).
STUDNITZ, W. VON: Glukagontest und Phäochromozytomdiagnostik. Schweiz. med. Wschr. **100**, 1023 (1970).
STUDNITZ, W., KÄSER, H., SJOERDSMA, A.: Spectrum of catechol amine biochemistry in patients with neuroblastoma. New Engl. J. Med. **269**, 232 (1963).
— LJUNGBERG, O.: Tyramine test and phaeochromocytoma. Acta med. scand. **182**, 341 (1967).
SWART, B.: Die röntgenologische Diagnose des Phaeochromocytoms. Dtsch. med. Wschr. **89**, 2335 (1964).
VENDSALU, A.: Studies on adrenaline and noradrenaline in human plasma. Acta physiol. scand. **49**, Suppl. 173 (1960).
ZIEGLER, W.: Die Bedeutung der Vanillin-Mandelsäure (VMS) für die Diagnostik des Phaeochromocytoms und für die Untersuchung des Katecholamin-Stoffwechsels. Helv. med. Acta **27**, 647 (1960).

IX. Testis

A. LABHART

With Contributions by

CHR. HEDINGER, G. KISTLER, J. MÜLLER,
A. PRADER, G. TÖNDURY, and M. ZACHMANN

A. Historical Dates

1677 HAM and v. LEUWENHOEK discover the spermatozoa.
1849 BERTHOLD shows that the capon recovers the feathers of a cockerel after reimplantation of the testicles, thus proving a humoral effect of the gonads.
1889 BROWN-SÉQUARD, experimenting on himself, demonstrates the rejuvenating effect of testicular extracts, thereby generating interest in endocrinology.
1911 PÉZARD succeeds in producing an effective testicular extract.
1931 Androsterone, the first crystallized compound with an androgenic effect, is extracted by BUTENANDT from male urine and
1934 clarified in its chemical constitution.
1935 LAQUER isolates testosterone from testicular tissue. Clarification of the constitution and partial synthesis of testosterone by RUZIČKA and WETTSTEIN and by BUTENANDT.
1942 KLINEFELTER, REIFENSTEIN and ALBRIGHT describe the syndrome called after the first author.
1958 FORD, JACOBS, and LAJTHA discover that this syndrome is caused by an anomaly of the chromosomes.
1968 BRUCHOVSKY and WILSON discover the conversion of testosterone to 5α-dihydrotestosterone (i.e. a more active androgen) by target organs. At the same time, ANDERSON and LIAO discover selective nuclear receptors for 5α-dihydrotestosterone in the prostate.

B. Embryology, Gross Anatomy and Histology

G. TÖNDURY and G. KISTLER

Embryology. In the human embryo about 2.5 mm in length, the primordial germ cells (gonocytes) are situated in the wall of the yolk sac, close to the root of the allantoic diverticulum. Whether they originate in the yolk sac endoderm in the pre-somite stage, or are derivates of the overlying splanchnopleuric mesoderm is still a matter of dispute. From the wall of the yolk sac, the gonocytes, which contain a large, vesicular nucleus and abundant cytoplasm, migrate through the mesenchyme of the dorsal mesentery into the epithelium and the mesenchyme of the gonadal anlagen. The primordia of the sex glands become light microscopically recognizable, in the embryo about 4–5 mm in length, as thickenings of the coelomic epithelium (= genital ridges) on the medial aspect of the anlagen of the mesonephros. The genital ridges extend first from the 6th thoracic to the 2nd sacral segment. The cranial and caudal portions regress rapidly and only the parts belonging to the upper three lumbar segments develop further. In this region, the coelomic epithelium becomes first highly columnar and then stratified. Its basement membrane disappears and cords of epithelial cells (= primitive sex cords) invade the underlying mesenchyme (Fig. 1). At this developmental stage, the primordial germ cells, which stain selectively with the naphthyl alkaline phosphatase reaction, are already present in both the sex cords and the mesenchyme surrounding them. The gonadal primordia are thus formed from cells of three different sources, the coelomic epithelium with its underlying mesenchyme, which are both of mesodermal origin, and the primordial germ cells, which migrate secondarily into the primordia. In the first six embryonic weeks, the gonadal anlagen of both sexes are morphologically indistinguishable (sexo-neutral stage) and mesonephric and paramesonephric ducts are both intact.

During the 7th week of development (length of embryo about 18–20 mm), the *testis* becomes identifiable as such. Proliferating mesenchymal cells separate the outgrowing, still compact sex cords from each other. At about 25 mm, a layer of fibrous connective tissue, the *tunica albuginea*, forms peripherally, just beneath the

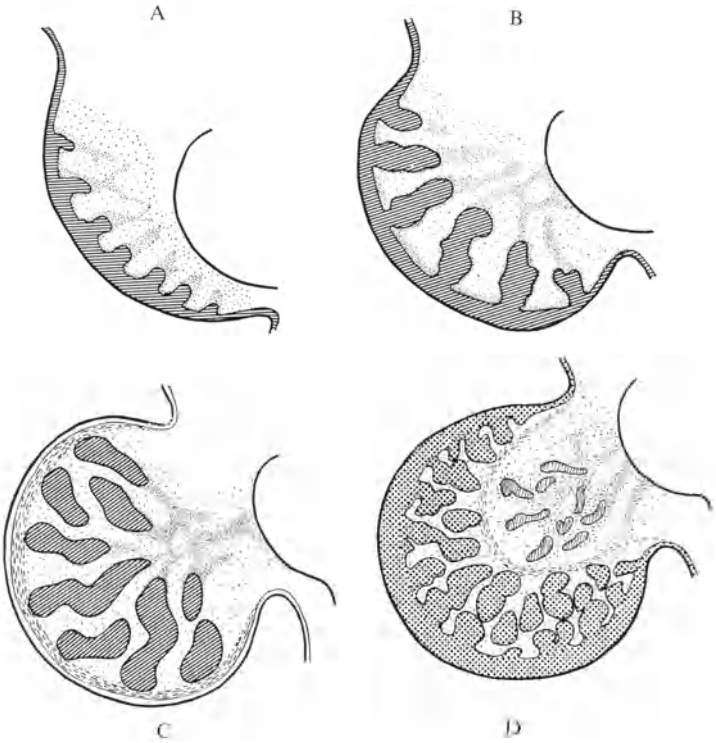


Fig. 1A–D. Diagrammatic representation of gonadal development. A) Proliferation of the coelomic epithelium in the region of the gonadal ridge. B) Development of the primitive sex cords, sexo-neutral stage. C) Development of the testis: Peripherally, the tunica albuginea develops and separates the medullary cords from the coelomic epithelium. Centrally, the outgrowing cords form the epithelium of the rete testis. D) Development of the ovary: Proliferation of secondary cords from the coelomic epithelium. A rudimentary tunica albuginea separates the cortex from the medullary portions of the anlage, which contain the rete ovarii. (Modified after R. K. BURNS, 1955)

germinal epithelium. The sex cords are thus separated from their original matrix. All primordial germ cells are successively taken up into the epithelial cords. Outgrowth of the cords leads to the formation of the *rete testis* in the gonadal mesentery (= the mesorchium). Finally, canalization of the cords gives rise to the *seminiferous tubules*. Their walls are formed by *sustentacular cells* (Sertoli cells), among which the primordial germ cells are dispersed.

The central parts of the epithelial cords, which form the rete testis, differentiate rather late into tubules (when fetuses are about 60–90 mm long). Some of these tubules contact mesonephric tubules which lose their glomeruli and become the *ductuli efferentes*. These ductules connect the rete testis to the mesonephric duct, which later becomes the *vas deferens*. The paramesonephric duct degenerates.

The *interstitial cells* (Leydig cells) differentiate from mesenchymal elements of the testicular stroma. In the early fetal stages (3rd to 5th month) these cells increase rapidly in number and display marked metabolic activity (production of fetal sex hormones).

Although the *chromosomal* sex of an individual is already determined at the stage of the fertilized ovum, the sexual differences in the gonads become morphologically detectable only after the 7th embryonic week. This still poorly understood differentiation, which is apparently not determined by the chromosomal sex of the immigrating primordial germ cells, establishes the *gonadal* sex of an embryo. *Genital* sex, finally, is highly influenced by hormones. It is established during the third month of gestation, when the sex ducts and external genitalia assume their specific masculine or feminine character.

The sex-specific differentiation of the gonads is a gradual process. In most mammalian species, the development of the testis seems to be determined as soon as the stage of the morphologically undifferentiated primordium, whereas that of the ovary is not. When a gonad at the sexo-neutral stage is transplanted from an experimental animal into the kidney of an adult specimen the genetically male gonad continues to develop normally. The differentiation of the genetically female sex gland, however, varies

considerably. Thus, in the rat embryo, the anlage of the testis is definitely determined by the 12th day, whereas the primitive ovary reaches a similar developmental stage only on the 15th to 16th day. From the 17th day onwards both male and female transplants develop typically (TORREY, 1950).

The androgenic *hormones* produced by the embryonic, differentiating testis are morphogenetically much more potent than the embryonic sex hormones formed in the ovary. Thus, in ovariectomized female rabbit fetuses, the genital ducts and the external genitalia develop more or less normally. However, in male rabbit fetuses castrated at the sexo-neutral stage, the mesonephric ducts degenerate and the paramesonephric ducts persist. In addition, the external genitalia assume a feminine appearance. The fetal testicular hormones seem, therefore, to stimulate the development of the mesonephric duct system and to inhibit the paramesonephric ducts.

The influence of the fetal *pituitary gland* on the differentiation of the gonads in humans is still poorly understood. The gonads and external genitalia of anencephalic newborn males, who usually have no pituitary gland, are normally not affected. It would thus appear that the hypophyseal gland does not play any major role in the development of the human sex glands.

Gross Anatomy. In the young adult, each testis weighs 10 to 16 g and measures approximately 4 × 2.5 × 3 cm. It is suspended in the scrotum at the end of the *spermatic cord* which comprises, besides connective tissue, the *ductus deferens*, (the excretory duct of the gland) and the blood vessels and nerves supplying the organ. The left testis usually hangs rather lower than the right one. The *epididymis* is closely applied to the posterior margin of the testis. It overlaps the lateral surface and the upper pole of the gland.

The testis is enclosed in a thick capsule of inelastic connective tissue, the *tunica albuginea*. On the posterior aspect of the gland, connective tissue of the tunica extends into the testis as the *mediastinum testis*. From here, incomplete septa project radially towards the tunica albuginea, thus dividing the testicular parenchyma into about 200–300 pyramidal lobules, the *lobuli testis*. Their apices converge upon the mediastinum. The innermost layer of the tunica albuginea, the *tunica vasculosa*, is rich in blood vessels. It is continuous with the loose connective tissue which forms the interstitium of the testicular lobules.

During its descent from the peritoneal cavity into the scrotum, each testis is accompanied by an outpocketing of the peritoneum, the *tunica vaginalis propria testis*, which consists of two layers. On the anterior and lateral surfaces of the testis, the inner, *visceral* layer is closely applied to the tunica albuginea. On the posterior aspect of the gland, the visceral layer is reflected and continuous with the outer, *parietal* layer. A cleft-like, serous cavity between the two layers, the *cavum serosum testis*, surrounds the gland except for the mediastinum testis and widens at its upper pole to form the sinus epididymidis.

The testis is supplied by a branch of the abdominal aorta, the internal spermatic artery. Some of its branches ramify in the tunica vasculosa, while others traverse the mediastinum testis and form the capillary plexus surrounding the seminiferous tubules. Other branches reach the epididymis, where they anastomose with the artery accompanying the ductus deferens. A number of veins emerge from the posterior aspect of the testis. Together with venous branches from the epididymis, they unite to form the *plexus pampiniformis*, which ascends along the spermatic cord. The lymph vessels of the testis supply the lateral and pre-aortic lymph nodes. The nerves accompanying the testicular vessels are derived from the 10th thoracic segment of the spinal cord. The sympathetic nerve fibers reaching the gland are probably vasomotor.

The *epididymis* is C-shaped. Its head lies on the superior pole of the testis, whereas its body is closely applied to the posterior margin of the gland. The lower, smaller part, the tail, contains the epididymal duct which increases in diameter to become the ductus deferens.

Histology. The histological appearence of the testis differs considerably according to the age of an individual. In the testicular lobules of a human fetus of about 60 mm CRL, canalized seminiferous tubules are still intermingled with some compact epithelial sex cords. Besides the supporting Sertoli cells, both contain large rounded *spermatogonia*, the successors of the primordial germ cells. The loose connective tissue surrounding the seminiferous epithelia contains numerous clusters and rows of polygonal cells with a large, spherical nucleus, the Leydig cells. The tubuli contorti of the testis of a 4-year-old boy (Fig. 2) contain supporting cells and spermatogenic cells at various stages of development. Only a very few immature sperms can be detected at this age, and the richly vascularized interstitium is completely devoid of typical Leydig cells.

In the adult, the lobuli testis consist of one to four convoluted seminiferous tubules, the *tubuli contorti*, which may reach a length of

60 to 70 cm. At the apices of the lobules, the tubules assume a straight course and unite to form 20 to 30 ducts about 0.5 mm in diameter, the *tubuli recti*, which open into the *rete testis*. This complex system of spaces in the connective tissue of the mediastinum testis is lined by a cuboidal epithelium. From the rete arise up to 20 convoluted, *efferent ductules* measuring about 5 mm in length and 0.6 to 0.8 mm in diameter. Together with the loose connective tissue surrounding them, they constitute the head of the epididymis. The ductuli efferentes gradually fuse into the highly tortuous ductus epididymis which is 4 to 6 meters long and forms the major constituent of the body and the tail of the epididymis.

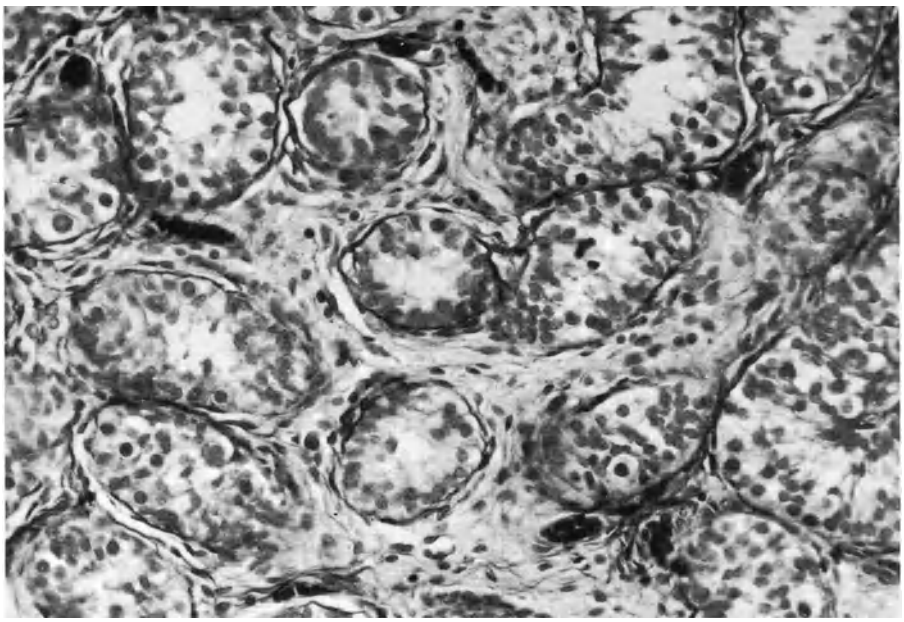


Fig. 2. Testis of a 4-year-old boy. Immature seminiferous tubules are surrounded by richly vascularized connective tissue. Note the complete absence of Leydig cells

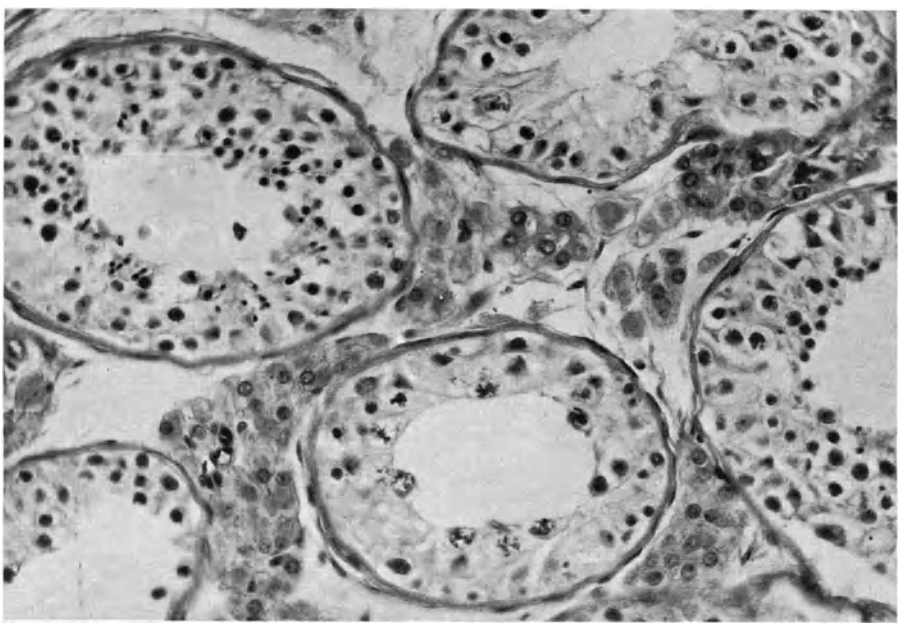


Fig. 3. Testis of a young adult. Seminiferous tubules with germ cells at various stages of development. The loose connective tissue between the tubules displays large clusters of Leydig cells

The tubuli contorti are lined by a stratified epithelium composed of sustentacular and spermatogenic cells. Each supporting or *Sertoli cell* rests on the basement membrane of the seminiferous epithelium. Its radially oriented, elongated nucleus contains a conspicuously large nucleolus. Numerous cytoplasmic, finger-like processes, whose outlines cannot be detected by light microscopy, extend upwards to the free surface of the epithelium. They are closely applied to those of neighboring Sertoli cells and to the spermatogenic cells. It is assumed that these cells, which usually contain lipid droplets and crystalloid inclusions (Charcot-Böttcher crystals), do not only support and protect the differentiating germ cells, but also play a role in their nutrition. Electron microscopically, the Sertoli cells are characterized by the presence of elongated mitochondria, often oriented parallel to the long axis of the cell, and by a moderately extensive rough- and smooth-surfaced endoplasmic reticulum. The cytoplasmic matrix also contains large numbers of microtubules and filaments. Beside lipid droplets and crystalloids, lipofuscin pigment granules are other typical paraplasmatic inclusions. The earlier opinion that the sperm cells are "embedded" in a syncytium of Sertoli cells has been found to be incorrect. Individual supporting cells are clearly separated by plasma membranes not only from each other, but also from the germ cells.

The sequence of events leading to the production of mature spermatozoa within the seminiferous epithelium is usually referred to as *spermatogenesis*. It can be divided into three phases, the first of which, the spermatocytogenesis, comprises the proliferation of the spermatogonia and the formation of the still diploid spermatocytes. In the second phase, meiosis, the spermatocytes undergo two maturation divisions. The resulting spermatids are haploid. In the third stage, spermiohistogenesis or spermiogenesis, the spermatids differentiate into the mature spermatozoa. The rounded or ovoid spermatogonia rest on the basement membrane of the convoluted tubules (compare Fig. 3). They display a spherical nucleus containing one to several nucleoli within a regularly dispersed chromatin substance. Their cytoplasm stains only faintly. The *primary* spermatocytes which arise from dividing spermatogonia first resemble their parent cell in size and affinity for dyes. After entering the first maturation division, however, their chromatin becomes condensed. The *secondary*, haploid spermatocytes resulting after the telophase of this division are definitively smaller than the primary ones. They rapidly enter the second maturation division, which leads to formation of the spermatids. Their differentiation into mature sperms is a very complex process involving the production of the acrosomal cap, the condensation of the nucleoplasm and the formation of the flagellum. A schematic representation of the mature spermatozoon is given in Fig. 4. In man, one complete cycle of spermatogenesis is estimated to be about two months.

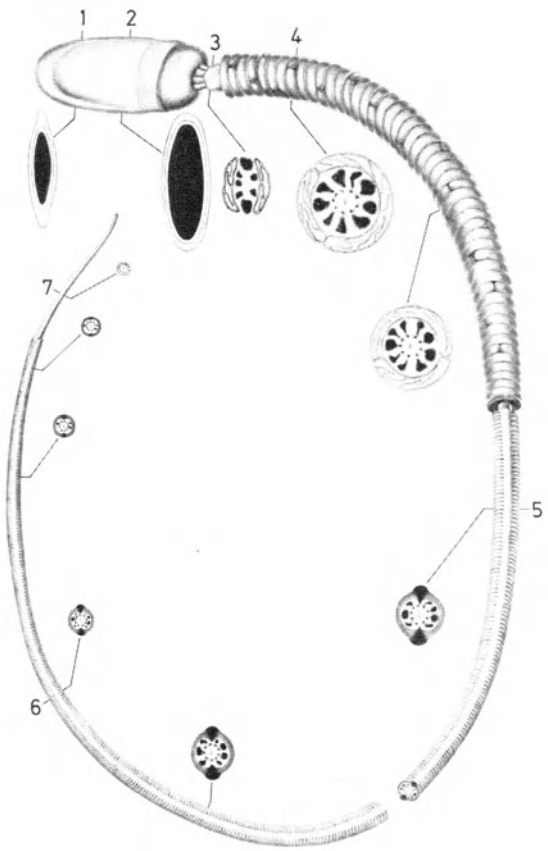


Fig. 4. Diagrammatic representation of a mature human spermatozoon. *1* acrosomal cap, *2* nucleus, *3* neck, *4* middle piece, *5* and *6* principal piece, *7* end piece. (Courtesy of Prof. D. W. FAWCETT, Boston)

The loose connective tissue surrounding the seminiferous tubules contains fibroblasts, mast cells and usually also a few macrophages and leukocytes. In addition, there are variously sized clusters and rows of epitheloid *interstitial cells*, the Leydig cells. They are diffusely dispersed throughout the lobules, often along the smaller blood vessels. The individual Leydig cells are polyhedral in shape and measure up to 25 µm in diameter. They usually have one large, spherical pale nucleus containing one or several deeply staining nucleoli. Binucleate cells are, however, also common. In most adult

human testes, the acidophilic cytoplasm of the interstitial cells contains faintly staining cristalloids which measure up to 20 µm in length and 1–3 µm in thickness. These so-called *crystals of Reinke* are protein in nature and their number varies considerably not only among different individuals, but also among the cells of the clusters. Electron microscopy reveals an abundant smooth-surfaced endoplasmic reticulum occupying the major portion of the cytoplasm in the Leydig cells, as well as a large Golgi complex. In contrast, only a few profiles of rough endoplasmic reticulum are usually seen. They are mainly situated close to the nucleus. Small lipid droplets and some lipofuscin pigment granules are regularly present. The mitochondria are large and similar to those present in the cells of the suprarenal glands, e.g. their cristae are vesicular or tubular in shape.

The sex hormone produced by the Leydig cells, *testosterone*, is not stored within incretory vesicles. It is assumed that the membranes of the smooth endoplasmic reticulum and of the Golgi apparatus contain the various enzymes needed by the cell for the synthesis of the hormone. The mechanism of its release into the capillaries surrounding the interstitial cells is still poorly understood.

C. Biochemistry

J. MÜLLER

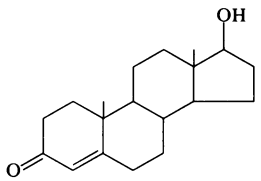
The testis is a gland with incretory and secretory functions. The interstitial Leydig cells produce androgens and estrogens; the spermatozoa arise from the seminiferous tubules.

1. Androgens

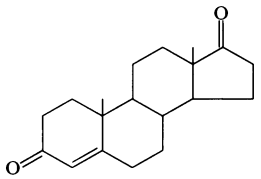
a) Human Androgenic Hormones

Androgens, i.e., the "man-makers", is a biological collective term for all substances which promote development of the male sexual characteristics. Almost all the known androgens are steroids and are therefore structural derivatives of cyclopentano-perhydro-phenanthrene (see p. 287 for the general chemistry of steroids). Natural androgens are C_{19} steroids without a side chain at C-17, but with two angular methyl groups. Androgenic activity is usually dependent on a double bond (between C-4 and C-5 or between C-5 and C-6) or on trans-linkage of rings A and B (H in the α-position at C-5). The type of substitution at C-3 and C-17 determines the androgenic potency.

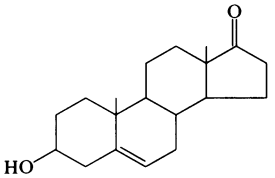
Among the human androgens so far discovered, *testosterone* shows the greatest activity in numerous bioassays and in the human, as far as can be assessed from clinical experience. In the nuclei of its target organs (prostate, skin) testosterone is enzymatically converted to di-



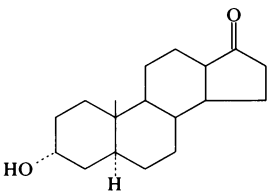
Testosterone



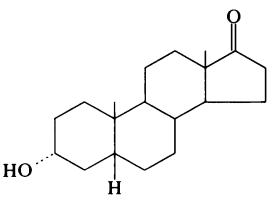
Androstenedione
(Androst-4-ene-3,17-dione)



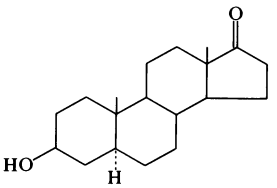
Dehydroepiandrosterone
(Androst-5-en-3β-ol-17-one)



Androsterone



Etiocholanolone



Epiandrosterone

Fig. 5. Important androgens and their metabolites in the human

hydrotestosterone (5α-androstan-17β-ol-3-one), which is a more active androgen than testosterone. Apparently, this activation in the target organ has to precede the biological action of testosterone (BRUCHOWSKY, 1968; WILSON, 1969). However, testosterone is the most important *circulating* androgenic hormone in man. Androstenedione, dehydroepiandrosterone and androsterone are 5 to 20 times less active than testosterone. Epiandrosterone possesses only a very low activity, and etiocholanolone none at all (DORFMAN and SHIPLEY, 1956). In contrast to testosterone, its metabolite, androsterone, causes a fall in cholesterol, phospholipid and triglyceride levels in the blood. Etiocholanolone causes fever and leukocytosis when injected intramuscularly in the free form. Impaired conjugation of this substance may be involved in the genesis of a special form of periodic fever, so-called "etiocholanolone fever" (BONDY, 1956).

Table 1. Normal values of human androgens. (Mean values after NEHER, 1968)

	Concentration in the peripheral plasma (μg/100 ml)	Blood production rate ("secretion" mg/24 h)
Men (20–80 years)		
Testosterone	0.65–0.81	6.1–7.8
Androstenedione	0.04–0.15	0.5–1.4
Dehydroepiandrosterone	1.3	3
Dehydroepiandrosterone sulfate	70–150	6
Women		
Testosterone	0.035–0.18	0.14–0.8
Androstenedione	0.1–0.3	3.4
Dehydroepiandrosterone	1.0	2
Dehydroepiandrosterone sulfate	50–100	6
Boys (4–9 years)		
Testosterone	0.04–0.06	
Androstenedione	0.12	
Girls (3–9 years)		
Testosterone	0.02	
Androstenedione	0.03	

As shown in Table 1, the concentration of *dehydroepiandrosterone sulfate* in the plasma in both male and female is much higher than that of the other androgens. This substance is secreted by the adrenal glands in the conjugated form (BAULIEU, 1965). Its functional significance is not yet known. It possesses no androgenic activity, but it is possible, at least in theory, that it may be a potential precursor of active androgens.

Androgenic activity in the plasma of the male is due almost entirely to its content of *testosterone*. The plasma testosterone level is about 20 times lower in the normal woman than in the man, the level of androstendione is somewhat higher and that of dehydroepiandrosterone about the same, so that these two weaker androgens are relatively more significant. It can be definitely assumed from concentration differences between peripheral plasma and plasma from the spermatic, ovarian and adrenal veins, that all three hormones are formed in the testes, ovaries and adrenal cortex (HOLLANDER, 1958; GANDY, 1965; HUDSON, 1967). It is, however, very difficult to assess the contribution of the individual glands to androgen production, since the androgens are converted into each other in the periphery (see below). In the normal man, at least 90% of plasma testosterone reaches the blood as testosterone (HORTON, 1966). Plasma testosterone is about 7 times lower in the castrated than in the normal man (COPPAGE, 1965). We can therefore assume with certainty that in the healthy man the majority of testosterone originates directly from the testes. The mean normal level of total plasma testosterone for the adult male is in the range of 700 ng/ml (see Table 1, p. 449) with a diurnal variation due to variation in production rate. There is a morning peak which is connected with fluctuations during sleep and rises in the testosterone level associated with periods of REM or paradoxical sleep (EVANS, 1971).

b) Biosynthesis and Production

The biosynthesis of androgens and estrogens in the testes proceeds in the same way as in the adrenal cortex (see p. 290 and Fig. 3). Cholesterol, which can also be fully synthesized from acetyl-coenzyme A in the testes, is probably an obligatory intermediate. A possible route of synthesis is via pregnenolone-progesterone-17α-hydroxyprogesterone to androstendione and testosterone. A second process involves conversion through 17α-hydroxypregnenolone to dehydroepiandrosterone and from there to androstendione. Numerous experiments *in vitro* and *in vivo* show that both biosynthetic pathways are possible in the gonads and adrenals, and it has never been ascertained that one process is more important than the other. Nevertheless, the 17α-hydroxypregnenolone-dehydroepiandrosterone route seems to predominate during fetal life. Synthesis of dehydroepiandrosterone sulfate from cholesterol sulfate and its possible conversion into active free androgens are probably only of secondary biological importance.

Assessment of the secretion of androgens from the gonads and adrenals is handicapped

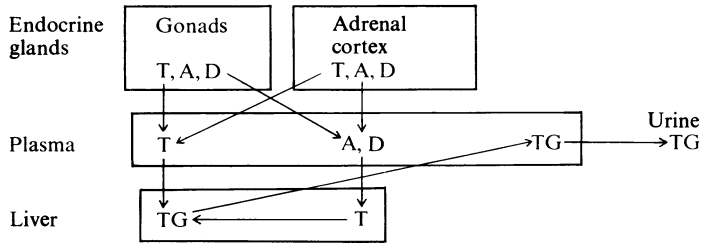


Fig. 6. Origin of testosterone in plasma and urine. (After LIPSETT and KOREMAN, 1964.) (T = testosterone, TG = testosterone glucuronide, A = androstenedione, D = dehydroepianandrosterone)

by the fact that circulating androgens can be converted into each other in the periphery. Thus, reversible conversion of testosterone into androstendione, and of dehydroepiandrosterone sulfate into dehydroepiandrosterone is possible, while dehydroepiandrosterone can be peripherally converted into androstenedione (VAN DE WIELE, 1963). According to HORTON (1966), 60% of the circulating testosterone in the woman, and less than 10% in the man, originates from circulating androstenedione. Conversely, 40% of the plasma androstenedione in the man arises from the circulating testosterone. It is still not known which organs are involved in these conversion processes. The role of the liver, in particular, is very obscure. All the reactions described above can occur in the liver, but the majority of testosterone formed in the liver is immediately and irreversibly further metabolized and conjugated. Labeled androstenedione given orally is therefore converted largely into testosterone, but only 2% of it is released into the peripheral circulation as free testosterone (HORTON, 1966). This explains why the so-called testosterone-urine production rates in the woman (estimated from the specific activity of testosterone glucuronide in the 24-hour urinary output after an intravenous injection of tracer doses of radioactive-labeled testosterone) are many times higher than testosterone-blood production rates (calculated by multiplying the metabolic clearance rate of infused labeled testosterone by the plasma testosterone concentration). In the man, however, this difference is insignificant.

c) Transport

Like glucocorticoids (see p. 291), the majority of unconjugated androgens also circulates loosely bound to plasma proteins. In man, 1.4% of testorone, 4.8% of androstenedione, and 2% of dehydroepiandrosterone are in a free, dialyzable from (RIVAROLA, 1968). The precentages of the free forms of these three androgens in the woman are 0.9, 2.7 and 2 respectively. They are partially bound to albumin, which shows a greater affinity for androgens than for glucocorticoids (DAUGHADAY, 1960) and has a practically unlimited binding capacity. It also appears that testosterone, and possibly also dehydroepiandrosterone, can also combine with a special β-globulin (MERCIER, 1966; VERMEULEN, 1969). In advanced pregnancy, the concentration of this testosterone-binding protein is raised, causing the proportion of free testosterone to fall to 0.2% (RIVAROLA, 1968), thereby preventing virilization despite increased plasma testosterone (MECKER, 1966).

d) Breakdown and Excretion

Testosterone circulating in the blood is very rapidly broken down and conjugated. The half-life as measured by the extractable radioactivity of the plasma after an intravenous injection of ^{14}C-labeled testosterone is on average 11 min during the first hour, and 100 min later on (SANDBERG, 1956). Only a slight amount of unchanged testosterone (less than 0.01% of the testosterone produced) is excreted in the urine (CAMACHO, 1964). About 1% of the circulating testosterone appears in the urine as testosterone glucuronide and 0.03% as testosterone sulfate. The greater part of testosterone is thus converted before it is conjugated and excreted.

It is impossible for us to discuss all the theoretically possible processes for the breakdown of testosterone, since, judging from their structure, there are about 60 different known substances which could be metabolites of testosterone (MOSEBACH, 1968). The following reactions are predominant in the catabolism of testosterone:

1. Isomerization or dehydrogenation of the 17β-hydroxy group;
2. α- or β-reduction of the double bond in ring A;
3. α- or β-reduction of the 3 keto-group.

In addition, there are numerous possible hydroxylations at different C atoms. Almost all the metabolites are conjugated with sulfuric acid or with glucuronic acid. Conjugated 17-

ketosteroids androsterone, etiocholanolone, and epiandrosterone are the quantitatively important metabolites of testosterone in the urine. About 40% of testosterone leaves the organism in this form (SANDBERG, 1956; WEST, 1951; SLAUNWHITE, 1957). The liver probably plays an important part in the catabolism of testosterone, but metabolism and conjugation are also possible in other organs.

The peripheral interconversion reactions of adrenal and gonadal androgens are described in section b. Because of these reactions it is impossible to draw valid conclusions about the type of androgen secretion and the organ from which it arises in healthy and diseased subjects from estimations of single 17-ketosteroid fractions. Even testosterone glucuronide is not exclusively derived from circulating testosterone; it can also originate from androstenedione (CAMACHO, 1964). This metabolite is therefore representative of testicular testosterone secretion only in the normal man. Since the portion of the testosterone produced which is excreted in the urine as testosterone glucuronide can vary between 0.15 and 1.88%, these figures are only of very limited value.

2. Estrogens

On average a man excretes about 10 μg estrogens in the urine in 24 hours, which is less than the woman does. The average daily production of estradiol in normal young men was calculated to be 70 μg (LIPSETT, 1966) according to the specific activity of estradiol glucuronide and estrone glucuronide in the urine collected after intravenous injection of ^{3}H-labeled estradiol. According to BAIRD (1968), the average so-called blood production rate (see p. 450) of estradiol in normal men is 39 μg/d and that of estrone 165 μg/d. Mean plasma concentrations are: estradiol 2 ng/100 ml; estrone 6 ng/100 ml. Treatment with HCG causes estradiol production to increase to twice or four times the original values. Estrogen excretion decreases after castration. We can therefore assume that most of man's plasma estrogens are directly or indirectly derived from the testicles. In a case of testicular feminization, estradiol concentration was found to be 10 times higher in spermatic vein plasma than in peripheral plasma (FRENCH, 1965). However, according to BAIRD (1968, 1969), less than 5% of total circulating estradiol is secreted directly into the bloodstream. More than 80% are produced by peripheral conversion of secreted testosterone, and approximately 15% by conversion of secreted estrone. On the other hand, 80% of circulating estrone is directly secreted into the blood stream, probably largely by the testicles (KELCH, 1972) and to a lesser extent by the adrenal cortex.

D. Physiology

1. Site of Production of Testicular Hormones

In man, testosterone is produced by the Leydig cells and to a slight extent by the adrenal cortex or by peripheral conversion of androgen precursors (see p. 449). In isolated damage to the tubular apparatus (bilateral cryptorchism, X-ray radiation), androgen production by the testes is maintained. Tumors of the Leydig cells cause a great increase in estrogen and androgen production. Stimulation with LH or with chorionic gonadotropin (HCG) causes an increase in the number and size of the Leydig cells as well as in testosterone and estrogen production. Leydig cells are present in the fetus, disappear a few weeks after birth and reappear during puberty. Excretion of testosterone varies accordingly. Chorionic gonadotropin increases estrogen excretion at the same time as it causes hypertrophy of the Leydig cells. The increase in estrogens exceeds that in testosterone so that the increased estrogen excretion cannot be due solely to the conversion of testosterone into estrogens.

2. Pituitary Control

The testes are controlled by the anterior pituitary. The pituitary has a two-fold control over the gonads in both male and female, and there is now little doubt that these two gonadotropic hormones, FSH (follicle-stimulating hormone) and LH (luteinizing hormone) or ICSH (interstitial cell-stimulating hormone) are identical in both sexes. In the female FSH acts on the follicle, and LH controls development of the corpus luteum. In the male, the Leydig cells are clearly under the control of LH, whereas the precise role of FSH and LH in regulating the tubular apparatus is not yet clear. Fluorescein-labeled FSH has been shown to localize in Sertoli cells and labeled LH in Leydig cells. LH exerts a stimulating effect on the seminiferous tubules by increasing the local concentration of testosterone. The mean concentrations in the adult male have been found to be between 4.1 – 16.0 μIU/ml for FSH and 10.9 to 24.2 μIU/ml for LH (2nd IRP units), both determined by bioassay or immunoassay. These values tend to rise with age (for review see BURGER, 1972). Clinical research now has biological as well as radioimmunological meth-

ods at its disposal for the separation of FSH and LH (see p. 563, Chap. X). They are, however, used for research purposes only. For clinical evaluation of single cases the bioassay of the total gonadotropins in the 24-hour urine is usually used. The rule that the hormone of the secondary controlled endocrine gland inhibits release of the stimulating hormone in the hypothalamus or in the hypophysis ("feedback" mechanism) (see p. 29, Chap. X), applies to the testes, as to all glands regulated by the anterior pituitary. Recently direct proof that LH secretion is inhibited by androgens has been obtained. A decrease of about 90% in the testosterone production rate can be demonstrated following oral administration of androgens and can be prevented by administering HCG at the same time (LIPSETT, 1966). On the other hand, most authors have not observed suppression of FSH due to androgens; at least their influence on FSH is controversial. Estrogens, however, inhibit LH and FSH only in pharmacological doses. They might also exert a direct effect on the Leydig cells, in certain dosages diminishing testosterone production with no decrease in gonadotropins. The formation of a second testicular hormone, "inhibin" or "x-hormone" in the tubules has been postulated, however, as gonadotropin levels are increased not only after castration or failure of the Leydig cells, but also in clinical syndromes with apparently isolated damage to the tubules (e.g. Klinefelter's syndrome). FSH is invariably elevated, while LH may be normal and is related to the testosterone level. The nature of this tubular substance, however, remains obscure. Although FSH levels may be elevated in severe oligospermia or azoospermia, it is still controversial whether there is any correlation between sperm count or the number of germinal cells and FSH level. It has been proposed that germinal elements, their breakdown products, or a yet unidentified "independent testicular station" (LEONARD, 1972), possibly located in the Sertoli cells, is responsible for FSH secretion in the male (THIEL, 1972).

On the other hand, in hypergonadotropic diseases with mainly tubular damage Leydig cells may also be functionally inferior. It is unlikely that estrogens formed in the Leydig cells or arising from testosterone conversion produce inhibition, since blockade of the hypothalamic receptors with the anti-androgen cyproterone (see p. 473), leads to a rise in the gonadotropins.

A diurnal rhythm for LH and FSH has not yet been conclusively demonstrated. It cannot, however, be excluded, although a diurnal rhythm in plasma testosterone levels and testosterone production rates might be due to variation in liver clearance for testosterone with vascular supply and physical activity (LIPSETT, 1966). The observation of seasonal fluctuations requires further investigation.

Human chorionic gonadotropin (HCG) obtained from the urine of pregnant women or from the placenta is chemically similar to human LH (see p. 670) and has almost the same biological activity. Its half-life, however, is considerably longer.

HCG causes plasma testosterone and testosterone and estradiol production rates to rise several-fold in normal males. There is a rise not only of testosterone in the spermatic vein of the dog, but also of dehydroepitestosterone, progesterone, and androstendione, precursors of testosterone (EIK-NES, 1967).

The reader is referred to the reviews by EIK-NES (1964) and BUTT (1967) for the influence of HCG, LH, and FSH on the testis in animal experiments, and to the book edited by SAXENA *et al.* (1972) for their effects in the human.

LH has a central role in the regulation of androgen biosynthesis and secretion by the testis, similar to that of ACTH in the regulation of the adrenal cortex. Like ACTH, LH stimulates the steroidal secretion increasing the biosynthesis of steroids. It acts at an early stage, probably before pregnenolone. The main action is the splitting of the cholesterol side chain. Furthermore, it stimulates the incorporation of labeled acetate into the androgens and so favors *de-novo* steroidsynthesis. Cyclic AMP seems to be the intracellular transmitter for ACTH and also for LH.

The exact mode of action, however, is still relatively unknown.

It is not sure to what extent LH acts by effecting changes of carbohydrate metabolism and protein synthesis, which have been observed *in vitro* in slides of testis tissue under the influence of HCG. As for ACTH, there may be different, and in part independent, sites of action at rate-limiting points in the biosynthesis. Active centers for the gonadotropin molecule are also unknown. Disulfide linkages and the carbohydrate of glycoproteins appear to be essential for the activity (BUTT, 1967). Like ACTH, HCG acts on intact cells only and not on tissue homogenates. For hormonal control of spermatogenesis see p. 455.

3. Action of Testosterone

The total effect of testosterone can be deduced from the difference between boy and man.

Testosterone promotes development of the genitalia, the secondary sexual characteristics, the larynx, the musculature, growth and skeletal maturation. It has a formative effect on the psyche.

a) Effect on the Genital System

Growth and function of the penis, epididymis, seminal vesicles, prostate, and Cowper's and Littre's glands are dependent on testosterone, as are growth and pigmentation of the scrotum. Testosterone acts via the seminal vesicles and the prostate to increase the seminal fluid and its fructose, citrate and acidic phosphatase content. Receptors for androgens have been found in the cytosol as well as in the nucleus of cells from the rat prostate (FANG, 1969). Testosterone is selectively accumulated in the prostate and seminal vesicles and is converted there to 5α-dihydrotestosterone (TVETER, 1968; BRUCHOVSKY, 1968). It has, however, yet to be confirmed whether dihydrotestosterone has a predominantly hyperplasiogenic effect on the prostate and its metabolite, androstane-3β,17β-diol, stimulates its secretion (BAULIEU, 1968; TAMM, 1972). In addition, it has to be proven, whether the hypertrophic prostate contains 4 to 5 times more dihydrotestosterone than normal (SIITERI, 1970).

The influence of testosterone on the testes themselves is complex. Testosterone has a trophic influence on the tubules. In hypogonadotropic hypogonadism the tubules do not develop under the influence of HMG alone but only when it is combined with HCG (see p. 474), i.e. after pretreatment with testosterone. Tubular atrophy and decreased spermatogenesis after hypophysectomy can be avoided to a great extent, though not entirely, by testosterone administration. Hormonal regulation of spermatogenesis has not yet been definitively explained (see p. 447). Testosterone is at least essential for the normal amount of spermatozoa and for the seminal fluid. On the other hand, large doses of testosterone lead to reversible atrophy of the testes and to azoospermia in the normal male. The seminal tubules become smaller, spermatogenesis decreases and finally ceases completely, the germinal epithelium becomes partly necrotic and diminished, the tunica propria thickens, and the Leydig cells disappear. After treatment is discontinued testicular tissue is completely restored (testosterone rebound, see p. 486). This action of high doses of testosterone is due to pituitary inhibition since it can be prevented by administering gonadotropin at the same time.

b) Effects on the Secondary Sexual Characteristics

The thickness of the skin, its circulation, and its ability to produce pigment increase under the influence of testosterone. The pubic hair assumes the characteristic male rhomboid form reaching up to the umbilicus only under testosterone influence, whereas in the woman and castrated male, it has a horizontal upper limit. Body hair can develop under the influence of testosterone, but only if the end-organs are sensitive, which varies widely individually. In most races, there is growth of beard and moustache in the male. North American Indians, however, remain beardless in spite of normal testosterone production, and in mongolian races the beard is scanty. The hair above the forehead recedes on both sides in the fully mature male at the peak of testosterone production. Baldness can only occur in the presence of testosterone. Eunuchs never develop baldness. Castration, however, does not bring about regression of baldness. Secretion of the sebaceous glands increases under the effect of testosterone. Its quantitative estimation indicates the degree of androgen production (STRAUSS, 1963). Overproduction leads to acne. Apocrine sweat glands (odoriferous glands) in the axillae and genital region begin to function. The larynx grows under the influence of testosterone, and the Adam's apple is formed. The voice breaks.

Testosterone promotes longitudinal and periostal growth of bones giving the male skeleton its characteristic form. The shoulders become broad and the pelvis narrow and long. The male development of the musculature is also due to testosterone.

In the woman, and especially in the child, testosterone has a "virilizing" effect, i.e., male sexual characteristics appear: beard and moustache, body hair, growth of the clitoris, deepening of the voice, forhead baldness, and in the child early skeletal maturation. Libido usually increases. The minimum dose leading to definite signs of virilization shows very great individual variations. On average, intramuscular injections of 150–300 mg testosterone propionate within one month produce signs of virilization in women. Contrary to popular opinion, hypertrichosis and hirsutism can appear with considerably lower doses of different androgens and also with so-called "anabolics" (see Chap. XX).

c) Metabolic Effects

Testosterone promotes protein synthesis. This "anabolic" action is always connected with a certain androgenic activity. Synthetic steroids

with an anabolic effect equal to or stronger than that of testosterone but with less virilizing effects have been successfully produced. Sensitivity to the virilizing properties of anabolic steroids varies with age and species. Children in particular are very sensitive, but no direct conclusions can be made from animal experiments about virilizing effects in the human.

The anabolic action of androgens promoting protein synthesis can be demonstrated *in vivo* in the human and in intact animals. The retention of nitrogen, electrolytes, phosphate and sulfate increases under the influence of the anabolically active hormones. They are retained in the same proportion in which they occur in the protoplasm. Raised amounts of protein are deposited in the accessory sexual organs and in the liver, kidneys, and musculature. Here too there are wide species-specific differences related to the same tissue among mammals. In addition, the same type of tissue responds differently to the anabolic action in various parts of the body (KOCHAKIAN, 1957). Estimation of ribonucleic acids at the same time shows that sometimes hypertrophy and hyperplasia occur together and sometimes hypertrophy alone.

Increased protein synthesis due to testosterone can be demonstrated *in vitro* in tissue sections from the liver and kidneys. Incorporation of labeled amino acids is increased, especially in the tissue of the seminal vesicles, and increased absorption of sulfate into cartilaginous tissue can also be shown.

However, we are only just beginning to understand the mechanism of anabolic activity. Labeled testosterone is rapidly stored in the accessory sexual organs, and later in the liver and musculature where it is converted to dihydrotestosterone. The anabolic action, however, only comes into effect when the labeled testosterone is uniformly mixed with endogenous testosterone. There may be two stages in protein construction, enzyme synthesis followed by protein synthesis. Testosterone increases different enzyme activities of protein metabolism such as β-glucuronidase, d-aminoacidoxydase, arginase, zymohexase and the phosphatases. Glutamicacid dehydrogenase, which is crucial in the metabolism of amino acids, is inhibited by testosterone and other steroids, probably due to alteration of its tertiary structure. The connection between the changes in these enzymatic activities and protein synthesis has not yet been explained. Finally, it has been shown in sections of the seminal vesicles of infantile rats that testosterone promotes neither the transport of amino acids through the cell membrane nor their synthesis during different stages of protein synthesis. Its effect is rather to enhance the combination of soluble ribonucleic acid-amino acid complexes to microsomal ribonucleoproteinates; this may be a remote result of a released chain of reactions (WILSON, 1962). Testosterone administered *in vivo* promotes the capacity of the ribosomes isolated from the prostate to incorporate amino acids into proteins. This effect seems to occur via an increase in the template RNA or their connection with the ribosomes (WILLIAMS-ASHMAN, 1964). Testosterone facilitates the attachment of RNA to ribosomes in some way which has still not been adequately explained (MANN, 1964). RNA synthesis in the seminal vesicles is promoted, and this also influences growth (VILLEE, 1967).

As for most hormonal activities, the molecular-biological bases are still at the hypothetical stage.

The influence of testosterone on muscular metabolism is indicated by a decrease in creatine excretion and by an increase in creatinine excretion. Creatine excretion, and creatinine excretion in particular, are increased due to the excessive synthesis caused by oral methyl testosterone.

The weight of the liver, heart, kidneys, and in particular, the accessory sexual organs decreases after castration. Testosterone produces the opposite effect. The effect on the kidneys, the so-called "renotrophic" action, does not appear to differ from the general anabolic action of testosterone. Renal blood supply is promoted by testosterone propionate in the healthy human. The fact that testosterone or anabolic steroids can cause a temporary standstill in uremia, in acute renal failure with oliguria, and in isolated cases of chronic renal failure is not due to the renotrophic effect, but to the anabolic effect and decreased production of protein metabolites.

In rats, testosterone and other anabolically active steroids can prevent adrenal atrophy after ACTH failure (see p. 300). The mechanism of action is unexplained.

It has still not been decided whether and in what way testosterone influences carbohydrate metabolism.

Testosterone, like many other steroid hormones, possesses the property of retaining sodium and therefore water. This effect is, however, considerably weaker than that of adrenocortical hormones, but edema may be an untoward side effect during sexual hormone therapy in older patients.

Effect on Fat Metabolism. The well-known clinical fact that eunuchs are less predisposed to coronary heart disease and that men under 40

years develop this disease 10 to 20 times more frequently than women of the same age is in keeping with experimental observations that estrogens inhibit experimental atherosclerosis and that androgens elevate low-density lipoproteins in the plasma.

Effect on the Blood. In the sexually mature male the hemoglobin level is on average 20% higher than in women and in eunuchs. The hematocrit and erythrocyte counts show corresponding differences, while the precursors in the bone marrow are not increased. The granulocytes and thrombocytes are unaffected by androgens. In rats, thymus and bone marrow are partially depleted of short-lived lymphocytes by high dosages of testosterone (FREY, 1970). High doses of androgens over a fairly long period promote normal or disturbed erythropoiesis in the human and may even lead to abnormally high hemoglobin values and erythrocyte counts. Slightly hypochromic macrocytes appear in the blood, and erythoid hyperplasia develops in the marrow. The mechanism of this effect is not clear. Inhibition of the erythropoietin-neutralizing effect of estrogens is being discussed.

Testosterone is capable of neutralizing some effects of estrogens. This antagonism also occurs directly at the reacting organ without involving the pituitary. See Chap. XIX, p. 1021) for the influence of androgens on development.

d) Psychic Effects

Small amounts of testosterone (e.g. testosteronepropionate, i.m., 5 mg every other day) cause no demonstrable effects in endocrinologically healthy women and men. On the other hand, treatment with high doses of testosterone (more than 500 mg per month) such as are used in carcinoma of the breast and in osteoporosis, usually causes an increase in the sexual drive of the endocrinologically healthy mature woman, but great quantitative and qualitative variations are observed in the individual reaction. Usually the increase in libido is found to be unnatural and pathologic. Emotional changes of short duration, often presenting as euphoria, may also occur.

For the psychic effect of testosterone and adrenal androgens in hypogonadism see pp. 459 and 309 respectively.

4. Semen

The morphology and biochemistry of the semen, a suspension of spermatozoa and other elements in the seminal fluid, have been extensively examined, particularly from the aspects of physiology and veterinary medicine. Origin, regulation of the formation, purpose, and function of its constituents have been extensively reviewed (MANN, 1964).

a) Spermatozoa

The cellular component of semen consists almost exclusively of spermatozoa, single cells of spermatogenesis and a few epithelial cells from the seminal ducts and their related glands. The morphology of the spermatozoa is discussed on pp. 447 and 494. Hormonal regulation of the formation and maturation of spermatozoa is still not clear. FSH and testosterone, or normally functioning Leydig cells induced by LH are necessary for the production of a qualitatively and quantitatively fully normal semen.

From individual experiments on hypophysectomized men, it has been deduced that HMG or FSH is essential either for maturation from the stage of spermatids to spermatozoa (GEMZELL, 1964) or for total maturation of spermatogonia (MCLEOD, 1966). Testosterone or LH and normal function of the Leydig cells are also necessary for quantitatively normal semen production. The entire process of spermatogenesis is still possible, albeit in a restricted way, even in the absence of testosterone (JOHNSEN, 1967; MCLEOD, 1966). Blockade of testosterone with cyproterone leads to inhibition of maturation at the spermatid stage (NEUMANN, 1968). Finally, experiments on testicular tissue cultures from different species have shown that differentiation of the germ cells right up to the late pachytic stage of meiosis is possible without hormones (STEINBERGER, 1967 a) and that FSH must be administered *in vivo* for completion of maturation (STEINBERGER, 1967 b). Spermatozoa are motile and produce the energy necessary for movement by fructolysis of fructose from the medium. Glycolysis is usually an anaerobic process. Entrance of air inhibits the use of the substrate via the Pasteur effect and with it the energy production and mobility of the spermatozoa.

Aerobic breakdown of phospholipids may be another source of energy. Spermatozoa are passively conveyed into the conducting system of the epididymis, where they mature further and are stored. Spontaneous movement does not arise until the surrounding medium provides oxidizable sugar or oxygen, or secretions of the prostate and seminal vesicles have a specific or nonspecific activating effect (MANN, 1964).

b) Seminal Fluid

The seminal fluid, with an average volume of 3.5 ml makes up the major part of the semen

in man; according to the species, the volume of semen varies between 1 ml (ram) and 250 ml (bear) per ejaculation, with corresponding differences in concentration (MANN, 1964). It is produced mainly by the seminal vesicles and prostate. The contribution of Cowper's and Littrè's glands and of the seminal ducts to the volume is only slight. Seminal fluid is a means of transport for the spermatozoa, providing a nutritional reservoir for them at the same time.

Seminal fluid contains proteins, peptones, amino acids, cholesterol, lactic acid, citric acid, pyruvic acid, inositol and sorbitol, which arise mainly from the prostate. In addition to Na, K, Cl and phosphate ions, traces of ascorbic acid and glucose are present. There are also 1 000–4 500 µg/100 ml of fructose, produced in the seminal vesicles from glucose through sorbitol. The normal amount of seminal fluid and its fructose and citrate content are dependent on testosterone activity (MANN, 1964; MCLEOD, 1966). Refer to MANN (1964) for the other organic and inorganic components. The high zinc content is worth mentioning. The polyamine, spermin, stems from the prostate and is responsible for the characteristic odor; it forms crystals in the cooled semen. Prostaglandins E and F, unsaturated dihydroxymonoketo acids are formed in the human prostate and lungs. They stimulate contraction of smooth muscle and have an antilipolytic action. Their physiological significance is not known (see Chap. XV, p. 990). Apart from this, proteolytic and other enzymes are found in the seminal fluid. These include fibrinolysin and fibrinogenase, which are important for coagulation and liquefaction of the semen. Acid and alkaline phosphatases are also present (MANN, 1964). Hyaluronidase is an intracellular enzyme, whose content seems to be proportional to the sperm count. The function of these enzymatic systems is unknown. It is possible that hyaluronidase is of importance in dissolving and penetrating the cervical mucus. The number of sperm may affect the fertility of a semen because of the content of hyaluronidase in the sperm. The importance of the seminal fluid in fertility is still very little understood. Ringer's solution with glucose is just as favorable a medium for maintaining motility of the spermatozoa. The buffer systems in the seminal fluid may be of significance.

Semen coagulates after ejaculation into a thick fluid mass with tapioca-like granules and reliquefies in 10–30 min after contact with air. The importance of this reliquefication is not clear, although it appears to be connected with the activation of sperm motility. Clotting impedes the loss of semen from the vagina. Perhaps, however, the only explanation for this phenomenon is a phylogenetic one.

E. Hypogonadism in the Male

1. Definition

The term male hypogonadism implies all states of testicular hypofunction, including incretory as well as secretory forms of insufficiency. Hypogenitalism means under-development of the external genital organs. Eunuchism [from εὐνή (bed) and ἔχειν (hold), εὐνοῦχος (the "bed-protector")] usually implies the condition after castration, whereas eunuchoidism means a congenital or acquired gonadal insufficiency. We also apply the term in this sense. Others use eunuchism for severe forms of hypogonadism and eunuchoidism for milder forms.

2. General Symptoms of Androgenic Failure

Only androgenic failure becomes clinically apparent, whereas impaired spermatogenesis does not affect the configuration of the body. The type is largely dependent on whether androgen production by the testes has been absent from birth, whether puberty has not occurred, or whether androgens have failed subsequent to puberty (see Fig. 7). The full picture of eunuchism and eunuchoidism arises after early castration or in congenital anorchia. The detailed description of the types of eunuchism can be found in the classical literature about castrates (PELIKAN, 1876; KOCH, 1921; WAGENSEIL, 1953; WOLF, 1934; PITTARD, 1934).

The physical stature of a man castrated early in life lacks male sexual characteristics, but female features are not pronounced (Fig. 8). The character is therefore neutral and childlike. The penis retains its infantile size even in the full-grown man, the scrotum is small, flat and not shaped like a sack, and is unpigmented; the prostate is not palpable or is only the size of a hazel nut, and the seminal vesicles are not palpable.

The skin is delicate, thin, and of a waxy pallor due partly to diminished blood circulation right up to advanced age. Normal male pigment formation is impaired in the absence of testosterone. In hypogonadism, ultraviolet radiation produces a reddening, but no browning. This may, however, develop subsequently under the influence of administered testosterone. In old age, the skin of the eunuch becomes very wrinkled, and old eunuchs are characterized by their wrinkled appearance. The cutaneous appendage organs remain underdeveloped. Pubic and ax-

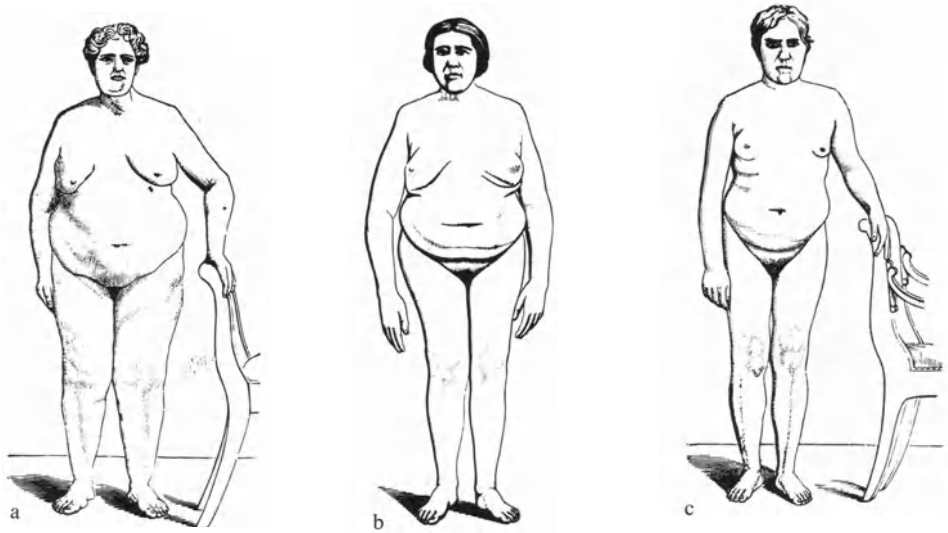


Fig. 7a–c. Influence of the timing of castration on the body build. a) Skopts who lost his genitalia at the age of 13: narrow shoulders, broad pelvis. b) Skopts castrated in his 6th year: broad pelvis. c) Skopts castrated at the age of 22: broad shoulders but narrow pelvis as in the normal man. (After PELIKAN, 1876)

illary hair may be completely absent, but scanty pubic hair of the feminine type is usually present due to the influence of adrenal androgens. Any other body hair is absent or remains at the lanugo stage. Beard and moustache may fail to develop, or there may be only a thin layer of lanugo on the cheeks and upper lip. In advanced age, as in the woman, a scanty beard is sometimes seen. The hair on the head is plentiful, fine in texture, and does not recede from the forehead on both sides. Eunuchs do not become bald, a fact which was already known to Aristoteles. The larynx in the castrated man is infantile and small; there is no Adam's apple, and it is not ossified. The voice therefore remains unbroken, and maintains its childish character. It is a childish soprano, not a woman's voice. It may become deeper with age.

There is a widespread opinion that castration leads to obesity. Statistical investigations, however, show that on average castrates and men with hypogonadism are no heavier than normal men. Nevertheless, fat deposition in eunuchs is prone to occur in characteristic parts of the body, such as the lower abdomen, hips, buttocks and mons pubis. Small fatty pads over the lateral parts of the eyelids are characteristic and lend a peculiar tired and sleepy appearance (BIEDL). The leptosomatic and pyknic habitus are otherwise no more peculiar to eunuchs than to the normal population. There is a thin tall type and a fat thickset type.

Growth of the bones and skeletal maturation are much influenced by testosterone. Failure of this hormone therefore causes both the eunuchoid stature and structural changes in the bones. The epiphyseal cartilages of the long bones fuse late, so that growth may continue to the 40th year. The epiphyses of the iliac bone may remain open for life. Delayed fusion of the

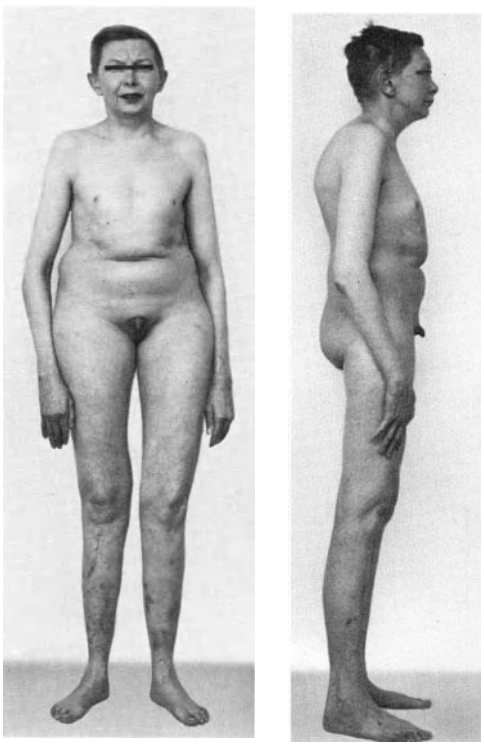


Fig. 8. 47-year-old man with congenital anorchia. Characteristic eunuchoid body shape. Upper body length : lower body length : half the extended arm length = 73 : 103 : 93. Small biacromial, wide bitrochanteric diameter. (Prof. A. SCHÜPBACH, Inselspital Berne)

epiphyseal cartilages of the long bones results in the eunuchoid height. The length of the lower part of the body and that of half the arm span exceed the length of the upper part of the body. The hypogonadal patient is a standing giant and a sitting dwarf (Fig. 8). The average height of eunuchs lies a little above the normal height of the corresponding population groups. The height is not parallel to the testicular insufficiency. Apart from the over-dimensional extremities, the skull and pelvis also demonstrate peculiarities. The three diameters of the skull are smaller, and the face is broader at the level of the orbits (Fig. 9). Protruding cheek bones give the hypogonadal patient the appearance of a mongoloid type and are reminiscent of the face of an Eskimo or an American Indian. The pelvis assumes a form varying between the narrow male and the broad female form. The bitrochanteric diameter is enlarged, whereas the biacromial diameter is decreased. Thus, the V-form of the trunk typical of the male is absent (Fig. 8).

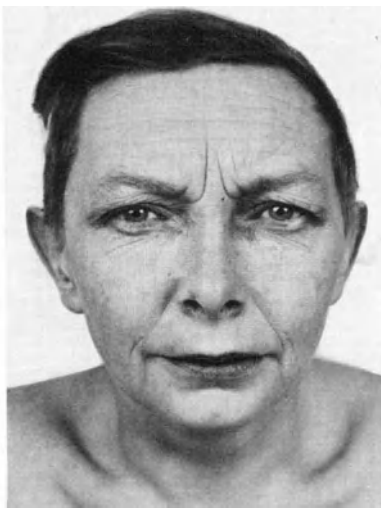
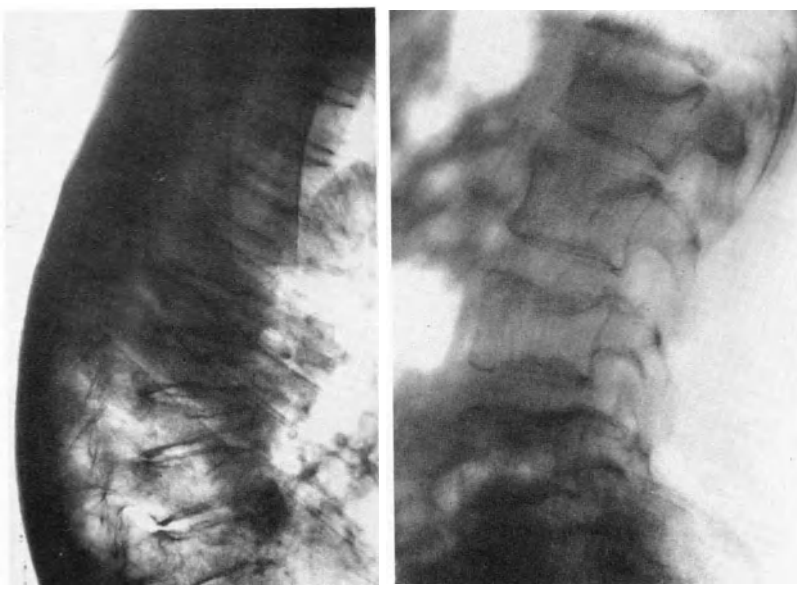


Fig. 9. 47-year-old man with congenital anorchia. Facial expression in extreme eunuchoidism: protruding cheek bones, fat pads over the lateral part of the eyelid. (Prof. A. SCHÜPBACH, Inselspital Berne)

Table 2. Symptoms of hypogonadism

Eunuchoid stature length of the lower part of the body and $^1/_2$ arm span > length of upper body (see Chap. XIX, p. 1022)
Underdeveloped, hypotonic musculature
Unbroken voice
Moustache, beard, body and axillary and pubic hair scanty or absent
Infantile genitalia small penis, testes, and scrotum

Failure of testosterone with loss of its anabolic action can lead to osteoporosis. Severe osteoporosis, with wedge-shaped vertebra and "fish vertebra" with vertebral infractions, is sometimes found in hypogonadal men after the 40th year (Fig. 10). According to Prokopius, the Byzantine



a b

Fig. 10. a) Extreme osteoporosis with anteriorly wedged vertebrae of the thoracic spine in a 55-year-old patient with idiopathic eunuchoidism. b) Osteoporosis with cod-fish vertebrae in a 46-year-old patient with congenital anorchia. (Prof. ZUPPINGER, Radiological Institute of the Inselspital Berne)

Field Marshall Narses was a eunuch with a hunchback. However, not all eunuchs develop osteoporosis. Only 2/3 of Chinese eunuchs have kyphoses (WAGENSEIL, 1953). NOVAKOWSKI (1952) found osteoporosis in 10 out of 26 cases of hypogonadism. Osteochondrosis appears to occur as frequently as osteoporosis and to present particularly frequently as Scheuermann's disease (juvenile kyphosis) with Schmorl's nodules on the vertebral column or the formation of wedge-shaped vertebra. Marginal ridges not yet fused with the vertebral bodies thereby become detached and so-called "apophysitis" develops.

Weakness of the connective tissue gives rise to flat feet and knock knees in male hypogonadism (Fig. 8). The joints are loose and are prone to arthrosis. Varicosis and hemorrhoids are common due to the yielding venous walls.

The male development of the musculature is absent even in hypogonadal males who do strenuous manual work. Increased creatine and creatinine excretions indicate impaired muscle metabolism.

Diminished protein metabolism leads to a slight fall in the basal metabolic rate. This is hypometabolism without concomitant hypothyroidism, although thyroid function may be reduced in a few cases (see p. 466). Castration does affect certain laboratory tests; hemoglobin, hematocrit, and erythrocytes are reduced by 10%, osmotic resistance is decreased, and the sedimentation rate is increased.

Although loss of testosterone after castration is compensated to a slight extent by the adrenal cortex, the gonadotropins are definitely elevated. FSH is increased to a greater extent, so that the FSH : LH ratio increases from 1.3 to 7 (BECKER, 1965).

Psyche. Postpubertal failure of androgens almost always results in a rapid loss of potency and sexual drive. Sometimes the drive is merely abated or recedes over the course of years. It seldom (in 1%) remains unchanged. The earlier castration is carried out the more pronounced is its effect on the psyche. It is mainly the physical drive which is influenced, and psychological sexuality may be retained as a need for love.

In congenital or prepubertal hypogonadism, there is psychic infantilism with impaired psychosexuality, inadequate character maturation, and to a lesser degree, impaired intellect, often associated with frequent primitive reactions and obstinacy – anxiety reactions and dependence on others. There is, however, also a physical "constitutional" form of infantilism, usually related to psychic infantilism, in which no endocrine disorder is demonstrable (BLEULER, 1964; LINDBERG, 1953).

Symptoms of the endocrine psychosyndrome are present: depression, moodiness, and passivity. Depression seems to be related to the preoperative personality and the social consequences of castration (BLEULER, 1955). The man castrated early in life and hypogonadal before puberty does not feel the loss of his sexual function for which he has never had any need. Male hypogonadism is therefore more often an incidental finding than a presenting symptom. In Albright's clinic this fact was known under the slogan "Forbes law": if a man complains of impaired sexual function, he is not suffering from a true endocrine disease but from a functional disorder. But even when a man castrated early does not suffer from his lack of sexuality, the high voice and eunuchoid appearance may cause social difficulties leading the patient to seek medical advice.

In contrast to early castration, later castration leads to little deviation from normal in appearance. Axillary and body hair and the beard, however, become sparser. The pubic hair seldom changes. Only the prostate invariably decreases in size. The later castration is performed, the less apparent are the physical changes (Fig. 7). See BLEULER (1964) for the psychiatric indications for and results of castration.

3. Investigation and Classification

The symptoms described above are more or less common to all forms of hypogonadism. However, only clinical examination and differentiation can reveal the etiology and pathogenesis. This is also of importance for the treatment.

Table 3 gives a survey of the full examination such as is possible in a hospital with laboratories and methods of endocrinological investigations at its disposal. Steps 1–3, inspection and palpation, simple X-rays and the simple semen examination, can, however, be undertaken by the general practitioner. It is possible to decide whether hypogonadism is present from these investigations alone. Sometimes their results may suggest classification into a sub-group. The differential diagnosis can, however, only be made with the aid of estimation of the gonadotropins, sex chromatin, and testis biopsy. Secondary sexual characteristics usually allow a good estimation of the 17-ketosteroid excretion, particularly of plasma testosterone. Estimation of 17-ketosteroid is only of importance in differentiating panhypopituitarism, in which the values are very low. Plasma testosterone gives more information than urinary testosterone, which

reflects only a small percentage of the total testosterone production.

Table 3. Clinical investigation for male hypogonadism

1. *Inspection and palpation*
   - Genitalia:
     - Length of the penis and its circumference (see Chap. XIX), hypospadia, epispadia
     - Size of testes (see Chap. XIX), consistency, tenderness
     - Size of the prostate and seminal vesicles
   - Stature:
     - Height, weight, upper body length, lower body length, arm span, constitution, fat pads
   - Hair distribution:
     - Pubic and axillary hair, moustache and beard growth body hair, hair on the head
   - Skin:
     - Texture, pigmentation, sweat and sebaceous secretion
   - Larynx:
     - Adam's apple, voice, pitch
2. *X-rays of the skeleton*
   - Skeletal maturation:
     - Hand and pelvis X-rays
   - Osteoporosis:
     - Thoracic and lumbar vertebra, lateral view
   - Pituitary size:
     - Sella turcica
3. *Examination of the semen*
   - Volume, viscosity, fructose content
   - Sperm count, motility
   - Morphological differentiation
4. *Sex chromatin, possibly karyogram* (see Chap. XII)
5. *Hormone determinations*
   - 17-ketosteroids
   - Gonadotropins in the urine
   - Testosterone in plasma and perhaps in the urine
6. *Testis biopsy*
7. *Tests for extra-gonadal pituitary functions* (see p. 122)
8. *Chorionic gonadotropin test for functional reserve of the Leydig cells* (see p. 474)

4. Different Forms of Male Hypogonadism

According to HOWARD (1950), there are two main groups: primary hypergonadotropic and secondary hypogonadotropic testicular insufficiency. We differentiate tubular, interstitial, and combined testicular insufficiency within each of the main groups (Table 4, Fig. 11).

The diagram is based on the assumption that the tubules are dependent on FSH, and the Leydig cells on LH. Testosterone probably has a local effect on maturation of the sperms, so that LH is also indirectly associated with spermatogenesis. The accuracy of the diagram has been largely confirmed since estimations of FSH and LH have been possible. For clinical purposes, when only the total gonadotropins can be clinically measured, isolated FSH or LH insufficiency has usually been recognized at testis biopsy.

Table 4. Classification of male hypogonadism

I. *Primary testicular insufficiency* (hypergonadotropic hypogonadism)
- A. Tubular and interstitial insufficiency
  1. Castration syndrome
     a) early
     b) late
  2. Congenital anorchia ("vanishing testis")
  3. Total atrophy
  4. Hereditary disorders and degenerative syndromes
  5. Anti-androgens ("chemical castration")
- B. Tubular insufficiency
  1. Exogenous damage
     a) Inflammation
     b) Heat (fever, injury to the spinal cord, bilateral cryptorchidism)
     c) α, β, γ rays
     d) Pressure
     e) Toxins
  2. Germinal aplasia (del Castillo syndrome), possibly identical to 1
  3. XXY trisomia, true chromatin-positive Klinefelter's syndrome and other chromosomal aberrations
  4. Chromatin-negative, "false" Klinefelter's syndrome
- C. Interstitial insufficiency
  1. Male climacteric

II. *Secondary testicular insufficiency* (hypogonadotropic hypogonadism)
- A. Tubular and interstitial insufficiency
  1. Isolated gonadotropin failure:
     "Idiopathic eunuchoidism" with low FSH
     Kallmann's syndrome
     Delayed puberty
     Hypothalamic damage
     Functional gonadotropin failure in starvation, cachexia, myxedema
  2. Panhypopituitarism, idiopathic or through pituitary destruction
  3. Adrenogenital syndrome
  4. Inhibition of FSH/LH by estrogen therapy or via impaired breakdown of estrogens in liver cirrhosis
- B. Tubular insufficiency
  Pubertas praecox
- C. Interstitial-cell insufficiency
  Testosterone therapy "Fertile eunuchs"?

a) Primary Testicular Insufficiency (Hypergonadotropic Hypogonadism)

In primary hypogonadism, gonadotropin excretion is raised to more than 3 mg/RP_2 per 24 hours. Normal values lie between 0.3 and 2 mg/24 hours, i.e. >4–10 IU FSH and >2 IU LH.

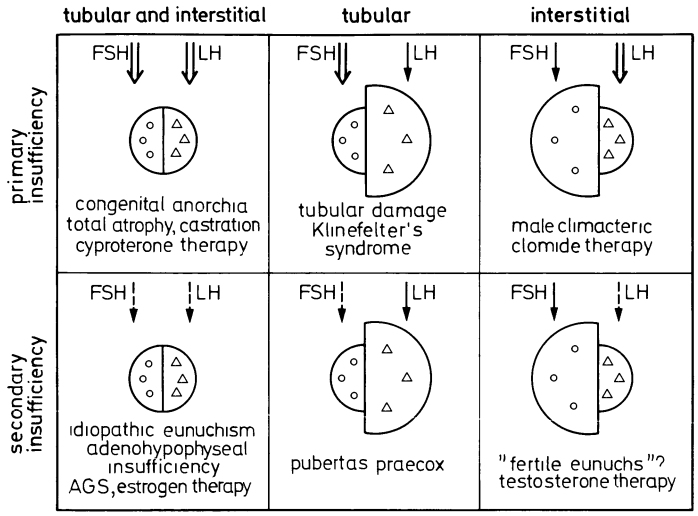


Fig. 11. Diagram of primary and secondary, tubular and interstitial testicular insufficiency

1 mg of the 2nd international reference preparation of HPG (1964) corresponds to 20–25 mouse units.

α) Tubular and Interstitial Insufficiency

1. Castration. The practice of castration was widespread in ancient times among the oriental peoples and has only fallen out of favor since the beginning of this century. The Greeks rejected the practice and it was only accepted in Rome during imperial times due to oriental influence. Castration was carried out to obtain harmless guards for harems, to tame war prisoners, to maintain the soprano voice in boys, and finally for religious reasons, in the Kybele cult, by the early Christians, and by the Skopts in Russia in the middle of the 18th century, on the authority of references in the New Testament (Matthew 19; Luke 23, 29). Although it is generally believed that loss of the male hormone leads to deprivation of energy and activity, eunuchs have achieved extremely high military, political, and religious honors (Narses, as Justinian's Field Marshall, Posides in imperial Rome, the patriarch Origenes).

Castration performed before puberty leads to the fullblown picture of eunuchism. The prostate and seminal vesicles atrophy, the thymus is hyperplastic, and characteristic changes occur in the anterior pituitary. The basophil and large chromophobe cells increase in number, and vacuoles and colloid appear in the basophil cells (the so-called castration or signet ring cells). Occasionally it even leads to tumor formation with enlargement of the sella (Bower, 1968). The 17-ketosteroid excretion is on average 7 mg/24 hours, corresponding to that produced by the adrenal cortex. It can be reduced to zero by 2 mg dexamethasone in the inhibition test.

Urinary excretion of testosterone-glucuronide decreases after castration, but by less than 90%, so that a compensatory increase in adrenal testosterone production is thought to be possible (Tamm, 1968).

Total gonadotropins are increased. It remains unexplained why FSH rises more, so that the FSH/LH ratio changes from 1 to 3–4 (Keller, 1968; Johnsen, 1972).

2. Congenital Anorchia, "Vanishing Testis". This diagnosis can be suspected when the scrotum is empty and the inguinal canal is free. The diagnosis can be confirmed and differentiated from bilateral cryptorchism only by surgical means. When the body shows signs of male sexual characteristics, there must have been a testis anlage which was destroyed during embryonic life. We therefore find "congenital anorchia" a more suitable term than "testis aplasia". "Prenatal castration" is no more explicit. The "vanishing testis" implies the pathogenesis better than "anorchia" (Abeyaratne, 1969). Complete absence of the gonads always leads to female sexual characteristics, regardless of the chromosomal sex. Stimulation with HCG does not effect any rise in plasma or urinary testosterone. Persistent extra-adrenal production of testosterone of unknown origin has been observed in patients with anorchia (Kirschner, 1970).

3. Total Atrophy. Total atrophy without differentiated cells can be the final stage of different pathological processes, such as orchitis, trauma, testicular torsion in particular, and impairment of vascular supply (defective orchidopexy, herniotomies). It may, however, also occur in the presence of positive sex chromatin and be an extreme form of Klinefelter's syndrome. Usually the etiology is unknown.

A small number of functioning Leydig cells often seems to be present, however; patients with testicular atrophy usually react to stimulation with HCG with a slight increase in testosterone excretion.

4. Hypogonadism in Hereditary Disorders and Degenerative Syndromes. In these cases there is predominantly primary damage to the testes, usually with involvement of the secretory and hormonal sections.

Steinert's Atrophic Myotonia. In this disease, which is inherited through a dominant autosomal gene, protein metabolism is impaired. In 80% of cases, the testes are atrophic in advanced age, whereas 70% of patients are fertile during early adulthood. Gonadotropins can generally be found in normal concentrations and are rarely elevated. The main feature revealed by testis biopsy is sclerosis of the tubules, and histological findings are remarkably similar to those in Klinefelter's syndrome, though the atrophy of the tubules is more homogeneous. Hypoplastic tubules such as occur in Klinefelter's syndrome are not seen. Leydig cell hypertrophy is also more homogeneous than in Klinefelter's syndrome. Differentiation is possible by the negative sex chromatin. Gonadotropin excretion is usually normal and plasma testosterone only seldom reduced, but HCG produces an inappropriate response (LIPSETT, 1966). Symptoms due to androgenic deficiency, gynecomastia and eunuchoid tallness are uncommon, in contrast to Klinefelter's syndrome.

Werner's Syndrome. This recessive sex-linked hereditary disorder first presents in later adulthood with premature baldness, if non obligatory small stature is not present. Other symptoms such as atrophy of subcutaneous fatty tissue, sensibility disturbances with cutaneous ulcers, cataracts, diabetes, arteriosclerosis and non obligatory primary secretory and incretory hypogonadism may develop. The high crowing voice is characteristic and cannot be explained by hormone failure. Incomplete forms of the illness may occur.

Rothmund's Syndrome. Poikilodermia, alopecia and cataract formation are usually associated with hypogonadism. The lack of pubic and axillary hair is probably cutaneous in origin and not due to androgen failure.

Laurence-Moon-Biedl Syndrome. This degenerative syndrome, which is only partly familial, consists of obesity, retinitis pigmentosa, debility, poly- and syndactylia. Primary or secondary hypogonadism can sometimes present as a facultative symptom.

Prader-Labhart-Willi Syndrome (Flour-Bag Dwarfs, HHMD-Syndrome, Myatonic Diabetes). Myatonia in very early infancy, small stature, obesity, imbecility, and diabetes of the stable adult type occurring at puberty are often associated with bilateral cryptorchism or anorchia and hypogonadism in boys. It has not yet been decided whether the hypogonadism is of the primary or secondary type. The definite, though inadequate increase in testosterone excretion in response to stimulation with HCG makes a purely primary hypogonadism appear unlikely (ZACHMANN, 1970). Secondary sexual characteristics are poorly developed in the adult. Beard and moustache are missing, pubic hair is of the female configuration, and the penis is infantile. Testes biopsies in the 3rd and 5th year of life show no germinal cells. Post-mortem findings in a 28-year-old included absence of spermiogenesis with slight tubular sclerosis and Leydig cells curiously rich in lipoid.

Hypogenitalism is present even in the male fetus. The scrotum is never definable from the perineal skin; it may be completely absent, or there may be a rudimentary suggestion of a scrotum in the form of coarse transverse folds in the skin with a median raphe. Often scoliosis, strabismus, and other degenerative symptoms are also present (Fig. 12).

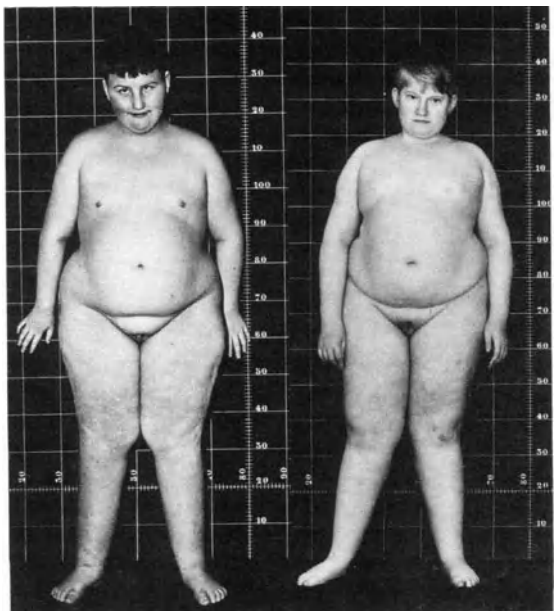
Cystic Fibrosis of the Pancreas. Males affected by this hereditary disease now sometimes reach adulthood. They are practically without exception sterile. The vas deferens is usually absent; the epididymis is malformed, the testes are often smaller than normal, and the germinal epithelium is diminished (KAPLAN, 1968).

The syndrome described by WEINSTEIN (1969), in which blind and deaf patients have small testes with hyaline tubuli and proliferating Leydig cells is also a form of primary hypogonadism, the gonadotropins being clearly increased. Hyperuricemia and hyperlipidemia seem to be constant symptoms in this syndrome. Plasma testosterone is in the lower normal range, and the secondary sexual characteristics are normally developed. The Leydig cells, however, are refractory to stimulation with HCG.

SOHVAL (1953) described cases of familial hypogonadism with gynecomastia, skeletal malformations, diabetes, and debility, which can probably also be classified into this syndrome. Histological findings of tubular changes are similar to those in Klinefelter's syndrome. There is tubular fibrosis and germinal aplasia,

the Leydig cells being proliferative in some parts and scanty in others.

See Chap. XII, p. 729 for other forms of more or less pronounced primary hypogonadism where male pseudohermaphroditism is the predominant clinical feature (testicular feminization; incomplete testicular virilization; Gilbert-Dreyfus syndrome; hereditary, vulviform or perineal hypospadia).



a b

Fig. 12a and b. 2 boys with the Prader-Labhart-Willi syndrome. a) 9-year-old. Oligophrenia, extreme obesity (76 kg with a heigth of 143 cm) especially in the region of the abdomen, the buttocks and the thighs of X-shaped legs, strabismus, acromicria, hypogenitalism. Occurrence of diabetes mellitus of the adult type at the age of 11. b) 17-year-old patient with oligophrenia, acromicria and subclinical diabetes, scoliosis, flabby obesity and dwarfism (140.5 cm at 67.5 kg), whose non-identical twin brother has a height of 176 cm and a weight of 54.5 kg, is normally built, of normal intelligence, and has a normal glucose tolerance (KspZ)

β) Tubular Insufficiency

1. Exogenous Damage to the Tubules. The tubules, and the germinal cells in particular, are more sensitive to exogenous noxious agents than is interstitial tissue. All degrees of tubular damage can occur, ranging from isolated failure of the germinal cells to complete fibrosis of the seminiferous tubules. Patients with tubular insufficiency appear normal since the androgenic action is intact, but the testes are usually too small. Apart from sterility, sexual function need not be impaired. The 17-ketosteroids and testosterone are normal or slightly reduced. On the other hand, gonadotropin and especially FSH excretion is nearly always elevated.

Inflammation. General infections spread to the testes relatively often. The first inflammatory reactions in the form of edema, hyperemia and small foci of inflammatory cells, possibly with abscess formation, usually arise in the interstitial tissue. The tubules are also involved at an early stage. In severe infections, such as smallpox, typhoid fever, and mumps, extensive necroses may develop. Fields of scar tissue with completely fibrotic tubules remain as a result from such types of orchitis.

These sequels are most commonly seen in association with mumps. The testes are not necessarily reduced in size. One or all tubules may be sclerosed. The interstitial tissue is penetrated by scars but usually contains well-maintained Leydig cells, in contrast to those found in Klinefelter's syndrome, in which the Leydig cells are abnormal and interstitial tissue contains less collagenous fibers. Mumps causes orchitis in one fifth of adult cases. This seldom occurs in childhood. Both testes are involved in only one sixth of cases. Atrophy follows in 40–60% of cases of orchitis due to mumps. Hypogonadism due to orchitis following mumps is therefore relatively uncommon. Since the mumps virus does not affect the immature testis there has been an attempt to use stilbestrol to produce reversible atrophy in adults with mumps. Control series, however, did not show any prophylactic effect. Cortisone, 300–200 mg, or prednisone, 60–40 mg per day, does not influence the course of the illness, as controls showed (KOCEN, 1961). The painful phase is, however, shortened. Only gamma globulins from convalescents have some prophylactic action (KABAL, 1963). Other viral infections which sometimes lead to orchitis with atrophy are chickenpox, dengue, phlebotomous fever, mononucleosis infectiosa, ECHO and Coxsackie-B viruses (CRAIGHEAD, 1962).

On the other hand, the incidence of luetic orchitis and luetic testicular scars has certainly been overestimated. Syphilis of the testis arises in two main forms: as interstitial and intratubular orchitis and as a gummatous inflammation. Although this can lead to total destruction of testicular tissue, diffuse orchitis can result in testicular fibrosis with predisposition for the tubules. The important clinical fact is that such cases of orchitis may progress undetected.

So-called granulomatous orchitis of unknown etiology is seen more often. It usually affects only one testicle and is characterized histologically by inflammatory foci of a granulomatous,

tuberculoid nature. There may be some connection with malakoplakia (BÜNZLI, 1968).

Tuberculous orchitis occurs in foci, hardly ever involves the whole testis and very seldom leads to hypogonadism without castration.

Thermogenic Damage. The destructive effect of heat has been observed in patients with pneumonia. It has been demonstrated experimentally that induced fever reduces the sperm count substantially in young men (STEINBERGER, 1959). Damage was seen in the rat testes at temperatures above 43° C.

The pathogenesis of tubular insufficiency in injuries of the spinal cord has not yet been explained. All degrees of damage to the germinal cells are found, often associated with gynecomastia. Since impairment of autonomic innervations can usually be demonstrated at the same time, it is possible that damage due to heat results from vasodilatation. On the other hand, the disturbance can be attributed to group IIB (Table 4).

Damage in bilateral cryptorchism is also due to the effect of the intra-abdominal temperature, which is 4° C higher than that in the scrotum. The tubules can show every degree of destruction. Often the Leydig cells are entirely spared (see p. 478). Frequently, however, undescended testes show primordial disorders in the germinal cells. Cryptorchism may also be caused by gonadotropin deficiency and can thus be classified as secondary testicular insufficiency.

It has been claimed that oligospermia may result from wearing tight underpants. Loose-fitting pants will at best contribute to an improvement.

Ionizing Radiation. α, β, and γ rays can lead to all degrees of damage to the tubular apparatus, depending on their intensity and the duration of exposure. Damage due to irradiation typically starts in the most sensitive elements, the spermatogonia, whereas the other stages of development are initially undamaged but cannot be regenerated. In contrast, the usual atrophy of the seminal epithelium affects the highly developed stages first. Radiation damage after atomic bomb explosions and radioactive exposure presents primarily as severe destruction ranging from minor to complete loss of germinal epithelium. From a few long-term investigations it can be concluded that the regenerative capacity, which takes some years to develop, is surprisingly great (HEMPELMANN, 1952).

Pressure Damage due to varicoceles and hydroceles leads to reversible azoospermia. Elevation of temperature may also have some effect. Pressure damage resulting from hematomas due to birth trauma can lead to permanent destruction (NOWAKOWSKI, 1956). Torsion of the testes, which is characterized by the persistence of pain on elevation of the testes, may result in infarction of the total testicular tissue and in extensive necrosis if the blood supply is not restored by surgery within a few hours.

Hypoxemia. Reversible oligospermia resulting from staying at extremely high altitudes is due to hypoxemia.

Germinal Aplasia (Del Castillo Syndrome, Sertoli-Cells-Only Syndrome). Patients with this syndrome seek medical advice because of sterility. Complete azoospermia is found and the physical findings are otherwise unremarkable, as is sexual function. Gonadotropin levels are usually elevated, but may also be normal. The testis biopsy shows typical findings: smaller tubules with fully mature Sertoli cells, with no cells of spermiogenesis.

DEL CASTILLO (1947) assumed that there was an embryonic developmental disorder in which gonocytes failed to migrate into the medulla. Occasionally, impaired growth and malformations such as occur in the Ullrich-Turner syndrome have suggested some connection with gonadal dysgenesis.

However, the histological findings resulting from injuries, X-rays, exposure to atomic bomb explosions (HEMPELMANN, 1952), as well as after treatment with cytostatics such as Myleran (BOLLAG, 1954), are the same.

Finally, autoimmunological reactions against testicular tissue produce a similar picture (MANCINI, 1964) (see p. 482). It is not yet known whether germinal cell aplasia is a single syndrome, or whether different causes can lead to the same condition.

2. Klinefelter's Syndrome (True, Chromatin-Positive Klinefelter's Syndrome, XXY Trisomia). Demonstration of the positive sex chromatin in 1956 and of XXY trisomia in 1958 showed, surprisingly, that the clinical picture of hypogonadism defined by KLINEFELTER and coworkers (1942) was due to a chromosomal aberration.

Klinefelter's syndrome is much more common than was assumed before it was possible to estimate the sex chromatin. Investigations of male newborns for sex chromatin gave an incidence of 1:1000, possibly even 1:400, among a population with lower intelligence. In schools for mental deficients the incidence is 1:42. Patho-anatomical findings of mixed post

mortems gave an incidence of 3.1:1000 (PASI, 1965).

Etiology and Pathogenesis. In contrast to the normal man with 46 chromosomes, the Klinefelter patient with a double X sex chromosome has 47 chromosomes. It is assumed that the disturbance usually arises in the mother during the first meiotic reduction due to nondisjunction of the sex chromosome, so that the ovum contains two X chromosomes instead of the haploid set of chromosomes with one X chromosome. It is possible that in a few cases of Klinefelter's syndrome the XXY trisomia is derived from the union of a normal ovum with an XY sperm resulting from non-disjunction in the father, in analogy to red-green color blindness, which is linked to the Y chromosome. Mosaic structures, i.e. cells with an XXY set of chromosomes in addition to cells with normal or XYY sets can occur in Klinefelter's syndrome. It is not yet possible to give the incidence of these.

As in mongolism, the other common condition caused by a non-disjunctional chromosomal aberration, the maternal age at the time of birth of Klinefelter patients is usually significantly higher than the average. It is conceivable that non-disjunction increases with the aging of maternal ova. On the other hand, there is no relation to paternal age. There is no familial incidence. However, other chromosomal aberrations, such as malformations of different types, occur more frequently among siblings of Klinefelter patients. The presence of the Y chromosome in mammals leads to the development of a male phenotype, but it is still unexplained why excess Y chromosomes can cause deviations in the development of the testes, degeneration of the tubular apparatus, disappearance of the germinal epithelium, inferior Leydig cells, and frequently gynecomastia as well.

KLINEFELTER'S original hypothesis that a second testicular hormone formed in the tubules, "X-hormone" or "inhibin", inhibiting FSH and development of the breasts in the normal man, has never been substantiated.

On the other hand, it has been shown that function of the Leydig cells is disturbed. Chorionic gonadotropin tests with estimation of estrogens (DECOURT, 1962), testosterone production rates, and plasma testosterone measurements (LIPSETT, 1966) show that testosterone production is in the lower normal range or reduced, and that it cannot be stimulated by HCG or only insufficiently. Testosterone synthesis is not blocked, but the abnormal Leydig cells need an increased LH stimulation to produce plasma testosterone levels approaching normal. LH is found to be elevated (BECKER, 1965), and the Leydig cells, which are already under maximum LH stimulation, cannot be further stimulated. Hyaline degeneration in the tubules is thought to be due to dysfunction of the Leydig cells (JOHNSEN, 1967).

Clinical Features. Before puberty, Klinefelter's syndrome can be clinically suspected only from the patient's lack of intelligence. The diagnosis, however, can be confirmed at any age by a positive sex chromatin finding, or the karyogram. The onset of puberty is delayed, and the testes maintain their infantile size of 2 cm^3.

Testis biopsies shortly before puberty show shrinkage of the germinal cells and developing tubular sclerosis (SIEBENMANN, 1958). During puberty, the size of the testis usually increases to about 4–6 cm^3 and decreases later with advancing tubular sclerosis.

In adults the testes are extremely small; their cut surfaces are intensively brown colored. The histological picture is characterized by an irregular sclerosis of the tubules with loss of the spermiogenesis and a relative increase of the Leydig cells (Fig. 13). The seminiferous tubules are atrophic; all stages of atrophy up to complete hyalinization of the tubules with total loss of the epithelium are found. However, especially in young patients, hypoplastic tubules and their remains can also be seen. The atrophic tubules may completely disappear so that finally only nests of Leydig cells remain. The Leydig cells show marked focal proliferation which manifests itself grossly in the intense brown discoloration of the cut surface. The polymorphism of the Leydig cells is very typical. Often they are markedly enlarged and possess a vacuolated cytoplasm. The nuclei also vary very markedly in size. With Feulgen's or hematoxylin stains the sex chromatin may be easily demonstrated. Gynecomastia is histologically characterized by an intense development of connective tissue whereas an epithelial proliferation is less conspicuous.

In adulthood, testicular atrophy is the one constant clinical finding. The body stature and secondary sexual characteristics can vary between the most severe eunuchoidism and a completely normal virile appearance (Figs. 14 and 15). As a rule, there are deviations in the body proportions, and the length of the lower half of the body is definitely greater than that of the upper body and half the arm span. Tallness and long legs appear before puberty and are thus not due to lack of androgens (SCHIBLER, 1971). There is often cubitus valgus and clinodactyly (ZUPPINGER, 1967). The pubic hair may be scanty or quite well developed, but

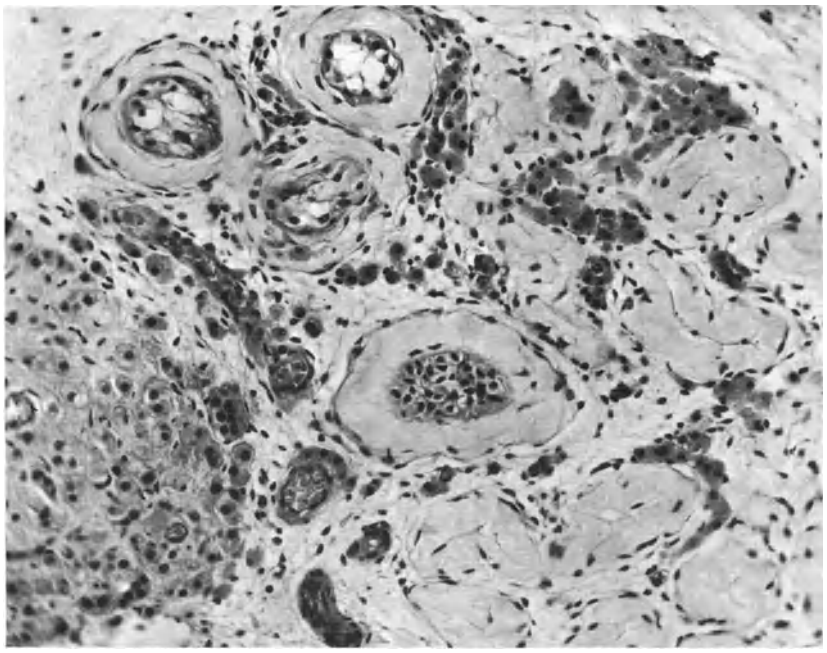


Fig. 13. MB 2996/53, 25-year-old man. Testes in Klinefelter's syndrome. Severe sclerosis of the tubules and focal hyperplasia of Leydig cells. HE staining, 180 : 1

it is of the feminine configuration with the horizontal upper border. The beard is usually thin, baldness seldom occurs, and the voice is masculine but rather high-pitched. The testes are almost always smaller than 5 cm^3, of normal or sometimes of reduced consistency. The prostate is of normal size or slightly smaller.

Two thirds of Klinefelter patients have gynecomastia which causes them to consult their doctor. It begins during or soon after puberty with development of pigmentation and swelling of the areolae. Hard glandular tissue surrounded by much fatty tissue then becomes palpable. In the majority of cases it is possible to differentiate the glandular from the adipose tissue by palpation with no difficulty. The gynecomastia can vary widely in degree, from the size of a hazel nut, with nonvisible, only palpable glands, to the size of the breast of a young woman.

Sexual function of Klinefelter patients is normal to begin with. Erection and ejaculation are unaffected. Sexual intercourse is usually entirely normal during early adulthood. Sterility is present almost without exception. A single case of a highly probable fertile Klinefelter patient has been reported. There was a sperm count of 200 000 cm^3 and the testes were as large as cherries (Warburg, 1963). Signs of involution, however, appear very early, often after the 35th year, obviously due to the inferior Leydig cells. Potency tends to decrease. Loss of androgens often results in a striking early osteoporosis. Thyroid hypofunction has been observed several times with no clinical manifestations and is probably of the primary type (Koller, 1955; Davis, 1963; Zuppinger, 1967). Diabetes, hyperlipidemia and obesity apparently occur more frequently than in the normal population (Zuppinger, 1967).

The majority of Klinefelter patients are slightly oligophrenic. Only a few cases are highly imbecile. On the other hand, not many are of average or above average intelligence. Pathologic EEG findings are common. In addition to the lack of intelligence, there is a certain psychological infantilism with an increased incidence of infantile deviations of sexual drive such as exhibitionism, transvestitism and homosexuality. Sexuality is, however, not very pronounced and decreases after puberty over the course of years and often disappears completely in the adult. Most of the patients show an endocrine psycho-syndrome with increased irritability and mood lability.

There may be an increased incidence of carcinoma (Lubs, 1962). This has been confirmed in the case of carcinoma of the breast, which occurs 70 times more frequently in Klinefelter patients than in normal males and about as frequently as in women (Jackson, 1965).

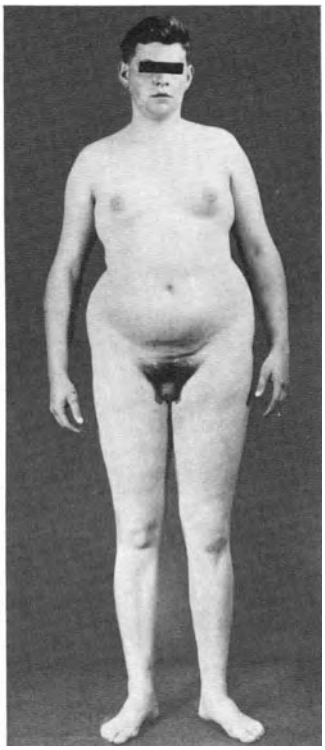


Fig. 14. 19-year-old, Klinefelter's syndrome, obese type with pronounced gynecomastia

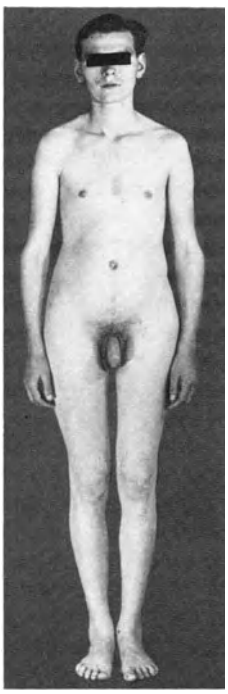


Fig. 15. 25-year-old, Klinefelter's syndrome, slender type, slight gynecomastia

Laboratory Findings. The sex chromatin in the buccal mucosal smear and the root of the hair is positive. Somewhat lower average values are found than in women, but there is no overlap with normal male values. Sex chromatin estimation from the nuclei of granulocytes is less suitable for the diagnosis of Klinefelter's syndrome since the drumstick appendage is found more frequently than in men, but less than in women. Examination of the ejaculate shows a reduction in semen volume. The fructose content is normal. There is azoospermia, and only very occasionally can single spermatozoa with impaired motility be seen.

The 17-ketosteroids may vary considerably. They are usually slightly reduced. Pregnanediol, pregnanetriol, estrone and estradiol are also decreased (GIORGIO, 1963). However the estrogen production rates are, increased (GABRILOVE, 1970). This suggests impaired conversion of androgens to estrogens. The rise due to puberty is retarded, while the fall due to age sets in soon after the 30th year. The low normal testosterone production can be stimulated by HCG only slowly and to a small degree, indicating a limited reserve of Leydig cells (LIPSETT, 1965; PAULSEN, 1968). Gonadotropin excretion is increased from puberty onwards and is usually over 4 mg IRP in 24 hours. FSH is almost always elevated; LH is increased or normal according to the plasma testosterone level (FRANCHIMONT, 1969; BECKER, 1965; KELLER, 1968). Normal values of gonadotropin have, however, been described in a few cases of confirmed Klinefelter's syndrome. Gonadotropin estimation is now superfluous for diagnosis when the sex chromatin is positive. The testis biopsy is characteristic (see p. 466) and, as a rule, can differentiate the true chromatin-positive Klinefelter's syndrome from other primary tubular testicular insufficiencies. It is no longer necessary to the diagnosis.

Differential Diagnosis. The true chromatin-positive Klinefelter's syndrome must be differentiated from other clinically and histologically similar forms with negative sex chromatin findings and different etiologies (so-called "false" Klinefelter's syndromes). Among others, hypogonadism in Steinert's dystrophic myatonia belongs to this group.

Multiple marginal chromatin bodies may suggest more serious irregularities in the set of sex chromosomes, such as XXXY, XXXXY, or XXYY, especially when combined with severe oligophrenia. These diagnoses can only be substantiated by a complete karyogram. Finally, the rare diagnosis of true hermaphroditism must also be considered, especially

when genital malformations or even hypospadia are present. Testicular biopsy is recommended in such cases.

Therapy. If the gynecomastia is severe it can only be corrected surgically. Only a small, hardly visible scar at the lower areolar margin remains when an experienced surgeon is available. When there are signs of androgen failure, substitution therapy with testosterone is indicated. Intramuscular injections of 250 mg testosterone-ester compound at 3-weekly intervals can improve waning potency, can prevent metabolic disturbances such as osteoporosis, and may cause deficient secondary sexual characteristics to develop. Substitution therapy may not be essential in early adulthood, but may become necessary with early involution in the fourth decade.

3. Hypogonadism with Tubular Fibrosis (So-Called "False" Chromatin-Negative Klinefelter's Syndrome). Primary hypogonadism with tubular fibrosis and negative sex chromatin is found somewhat less frequently. Disproportion of body stature, eunuchoid features and gynecomastia can be variably pronounced. The histological findings in the testes are characterized by less pronounced hyalinosis, and less impaired spermatogenesis than in true Klinefelter's syndrome, and diffuse instead of focal hyperplasia of the Leydig cells.

The etiology seems to vary. Familial occurrence has been described on different occasions.

Familial hypospadia with hypogonadism, as described by REIFENSTEIN (1947) and BOWEN (1965), also belongs in this group of syndromes. There is an external resemblance to Klinefelter's syndrome in addition to the hypospadia. In both syndromes the secondary sexual characteristics, i.e. eunuchoidism, are variably pronounced. Gynecomastia may also be pronounced or may be absent. The karyogram, however, is always normal, and the Y chromosome seems to be intact. The testes are less atrophic, and histologically there are atrophic tubuli with incomplete hyalinization and partly preserved elastics fibers, single Sertoli and germinal cells up to the spermatocytes. There are no morphologic alterations in the Leydig cells. There is azoospermia and sterility. The gonadotropin levels are usually raised though occasionally normal. Inheritance is either autosomal dominant with restriction to the male, or recessive and bound to the X chromosome.

The functional prepubertal castration in males described by HELLER, NELSON, and ROTH includes cases of primary hypogonadism of various etiologies.

4. The XYY Trisomia. The incidence of this syndrome is estimated at between 1 : 250 and 1 : 1000 male newborns (PFEIFFER, 1969; SERGOVICH, 1969). Alterations of the personality with aggressiveness and a tendency to criminal behavior, which were observed in the first cases examined (JACOBS, 1965), are not obligatory symptoms. Extreme height is characteristic of all these individuals. Up to now about 140 cases have been observed, 30 of them children. Differentiation into 4 subgroups might be possible; an additional Y chromosome can be found in normal, tall males, in tall criminal males, in tall males with hypogonadism or malformations of the external genitalia, and in tall males with malformations of other organs (HIENZ, 1969). Radioulnar synostosis seems to occur especially often (CLEVELAND, 1969).

There are no hormonal disorders in these patients: urinary and plasma testosterone is normal (ISMAIL, 1968; RUDD, 1968).

Growth hormone levels, fasting and after glucose load, are also normal. Tallness is not due to overproduction of growth hormone (NIELSEN, 1969). The chromosomes of parents and children of these patients are normal. The age of the parents is not different from that in the normal population. Chromosomal mosaics can occur.

γ) Interstitial Insufficiency

1. Male Climacteric. The menopause develops without exception in the woman, whereas the diagnosis of a climacteric is only seldom justified in the male. Strict criteria must be applied, and the diagnosis must not be made merely from age and subjective symptoms. In the woman, hormone production ceases abruptly. In the male, on the other hand, testicular function fails only gradually, if at all. Even in subjects over 90 years, the morphology of the testicular tissue may be completely normal (BÜRGI, 1959; DRY, 1967; NOWAKOWSKI, 1959). As testicular hormone production decreases, the body first tries to compensate by increased stimulation through gonadotropins. Whereas this compensatory stage is always of short duration in the female, the compensated state is the rule in the man, and manifestations of failure seldom appear. Climacteric failure shows a predisposition for men between 45 and 60, but it has been claimed to occur after the 25th year. Climacteric complaints in the male correspond to the typical symptoms in the woman: increased autonomic lability with hot flushes, sweating, palpitations, precordial pain, paresthesia, easy fatigability, nervousness, inability

to concentrate and depressive tendencies. The main complaint, however, is decrease in potency and sometimes in libido as well. All these complaints can, however, occur as a neurasthenic symptom complex without hormone loss. A definite rise in gonadotropin excretion is the only sure criterion. This corresponds to the findings in the female. The diagnosis ex juvantibus is simpler: climacteric complaints regress in response to a daily intramuscular injection of 25 mg testosterone propionate for 2 weeks, and they reappear when placebo injections are used instead of testosterone. The levels of 17-ketosteroids may be normal or slightly lowered. Their estimation is not conclusive. Estimation of plasma testosterone may be usefull for clinical purposes. Histological examination reveals a decrease in the number of Leydig cells and degenerative changes. The tubules are not affected, with the exception of occasional peritubular fibrosis. Spermatogenesis is only infrequently reduced (HELLER, 1944). Other authors have not observed histological changes despite elevated gonadotropin levels. See p. 475 for treatment.
for bioassay, <0.1 mg IRP/24 h. Urinary and

b) Secondary Testicular Insufficiency (Hypogonadotropic Hypogonadism)

The second main group, secondary hypogonadism, is recognized by the reduction or absence of gonadotropins. With prepubertal onset, the eunuchoid body stature is usually especially pronounced clinically.

α) Tubular and Interstitial Insufficiency

1. Hypogonadotropic Eunuchism (HELLER and NELSON), *Idiopathic Eunuchoidism with Decreased FSH* (HOWARD), *Isolated Gonadotropic Failure.* This disease is due to an isolated gonadotropin failure. The appearance of the patients differs little from that due to fully developed primary hypogonadism (see p. 476) (Fig. 18a). The size and consistency of the testes are the same in both conditions. They correspond to the testes of a boy before puberty, and have a volume of 1–5 cm^3.

The histological picture is similar to that of a boy's testis before puberty in different stages of development. In severe forms the seminiferous tubules form completely lumenless strands which enclose inactive germinal cells and undifferentiated Sertoli cells (Fig. 19a). The tunica propria is not thickened. Leydig cells are absent. In a somewhat advanced stage of development, the tunica propria may be thickened and contains only a small amount of elastic fibers. There are numerous spermatogonia in the tubules, and occasionally spermatocytes and differentiated Sertoli cells can also be recognized. Development of the Leydig cells is, however, deficient in these cases also. A thickening of the tunica propria can but does not always represent atrophy. It also arises in primary hypoplastic tubules and, in addition, with different hormone treatments (see p. 471). A thickened tunica propria can be indicative of many things; the etiologies are not yet satisfactorily explained. On the other hand, demonstration of elastic fibers permits certain conclusions to be made about the time of developmental standstill, since elastic fibers in the tunica propria do not appear until puberty. They may, however, disappear again with complete tubular sclerosis.

Hormonal Findings. The 17-ketosteroids vary between low (2 mg/24 h) to subnormal values (9 mg/24 h). Gonadotropin excretion is too low for bioassay, <0.1 mg IRP/24 h. Urinary and plasma testosterone rises quickly on stimulation with HCG.

Delayed puberty is of utmost importance in the *differential diagnosis.* It cannot be distinguished clinically from hypogonadotropic eunuchism, and therefore cannot be diagnosed with certainty before the 18th year (see Chap. XIX).

BARTTER and his team (1952) distinguished different forms of hypogonadotropic eunuchism, depending on results of treatment with chorionic gonadotropin.

In the first type the releasing mechanism of puberty is impaired. Development, once initiated with chorionic gonadotropin, continues spontaneously and the anterior pituitary produces gonadotropins from then onwards.

In the other form, function of the Leydig cells, and therefore development of the sexual characteristics, are only maintained as long as chorionic gonadotropin is administered. After withdrawal of treatment, the sexual characteristics regress again, but a higher degree of maturation is nevertheless often achieved by means of the treatment. Hypogonadotropic eunuchism is therefore due to complete inability of the adenohypophysis to produce gonadotropin, to a lack of the releasing hormones LRH and FSHRH, or to the absence of a mechanism for releasing hormone secretion. The etiology is unknown. Familial incidence has been reported. The pathogenesis of gonadotropin failure is largely obscure. Clinically, *hypothalamic hypogonadism* can only be diagnosed when other manifestations of hypothalamic failure, such as diabetes insipidus, are present at the same

time (see p. 32). Today, LHRH application can be of help in differentiating between pituitary and hypothalamic gonadotropic insufficiency (MARSHALL, 1972).

ROSEWATER (1965) described a type of hypogonadotropic hypogonadism with gynecomastia, descreased numbers of Leydig cells, and impaired maturation in the sperm. It is inherited recessively with the X chromosome or dominant autosomally and restricted to males.

2. Hypogonadotropic Eunuchism with Anosmia (Kallmann Syndrome). The combination of anosmia or hyposmia with eunuchism forms a special syndrome within hypogonadotropic hypogonadism. The syndrome was described by MAESTRE DE SAN JUAN over a hundred years ago and has only recently been recalled by KALLMANN (1944), DE MORSIER (1954), and NOWAKOWSKI (1961).

In addition to severe eunuchism including gynecomastia, as described in the previous section, the patient's sense of smell is usually completely absent or sometimes severely impaired from the time of birth.

Aromatic smelling substances, such as coffee, anis, asa foetida, are not recognized, whereas patients can identify substances such as ammonia, which stimulate sensory nerve endings of the trigeminal nerve in the nasal mucosa.

The sense of smell must be tested in every case of eunuchism. Patients pay no attention to this disorder and do not mention it spontaneously. Patho-anatomical findings include agenesis or defects of the olfactory nerves. Usually the anterior parts of the central olfactory system, i.e. bulbus, olfactory tract, and olfactory trigone, are involved, whereas the posterior parts (the anterior perforated area, parolfactory area and subcallosal gyrus) are not affected.

Atrophy of the hypothalamic nuclei tuberis is sometimes found, while in other cases it is not found despite serial sections.

LH and FSH excretion is similar to that in normal children and hypopituitary patients. Clomiphene does not usually stimulate LH excretion. HCG may or may not raise the low plasma testosterone levels, so that unresponsiveness of the Leydig cells has been described as a further factor of hypogonadism in this syndrome (BARDIN, 1969; SANTEN, 1973). LRF may increase slightly plasma LH, suggesting a hypothalamic disorder (NAFTOLIN, 1971).

The association of anosmia and gonadotropin deficiency can be plausibly explained as a developmental disturbance because of the close embryonic connections between pharyngeal and nasal mucosa, hypophysis, rhinencephalon and hypothalamus (see p. 27). The sex chromatin is negative and the karyogram is normal. The familial incidence of the syndrome suggests an autosomal dominant inheritance with incomplete expressivity (SANTEN, 1973). The gene leads in the female only to anosmia or hyposmia and cases with hypogonadotropic hypogonadism have been sporadic (TAGATZ, 1970).

The reader is referred to NOWAKOWSKI and LENZ (1961) for other forms of familial hypogonadism which are rather difficult to classify.

3. Hypogonadism in Malnutrition and in Hypothyroidism. The hypophysis, being an organ of adaptation in combination with the hypothalamus, limits production of certain hormones in hunger states and in severe illnesses. Gonadotropins fail, testosterone production decreases, and spermatogenesis regresses. In the undernourished calf, sexual maturation is delayed, the testes contain little testosterone, the seminal vesicles produce less fructose and citrate, but semen formation is less affected. These are most probably results of gonadotropin deficiency. However, the seminal vesicles of the undernourished animal show little response to testosterone (MANN, 1967). Long-lasting starvation states can lead to tubular and interstitial atrophy in the human (UEHLINGER, 1948). The syndrome of hypogonadism with gynecomastia has been observed in prisoners of war in Japan as well as in German concentration camps, and developed when feeding was restarted after a long period of malnutrition. Its pathogenesis is unexplained (see p. 488).

A special form of secondary hypogonadism of varying severity with normally large or enlarged testes, and with predominantly tubular insufficiency has been described in myxedema (DE LA BALZE, 1962). The gonadotropin-producing cells may be compressed and damaged by the hypertrophied TSH-producing cells. In contrast, an overproduction of gonadotropins was explained in hypothyroidism through feedback overlap, which leads to pubertal precocity (see Chaps. VI and XIX, p. 175, 1050).

4. Panhypopituitarism, Partial Pituitary Insufficiency. The clinical findings in this sub-group have been dealt with in the chapter on the hypophysis (see p. 97). The findings in the testes correspond to those in congenital pituitary dwarfism and in acquired pituitary insufficiency, as described above.

Hypogonadism in the true Babinski-Froehlich syndrome, so-called "dystrophia adiposogenitalis" (see p. 98), also belongs to this group. A gonadotropin deficit is usually also assumed to be present in the Laurence-Moon-

Biedl syndrome, although cases of primary testicular failure have also been known to occur.

5. Adrenogenital Syndrome (see p. 367). The AGS causes hypogonadism in both sexes. Paradoxically, hypogonadism due to atrophy or hypoplasia of both parts of the gonads develops in association with hypergenitalism, i.e., excessive development of the sexual characteristics. This is due to inhibition of the gonadotropins by the adrenocortical androgens and estrogens (see p. 366). When there is ectopic adrenal cortical tissue in the testes, these may appear to be enlarged and a mistaken diagnosis of carcinoma may be made (HEDINGER, 1954).

The histology of the testes is very similar to that in hypogonadotropic eunuchism: Leydig cells are absent, the lumens of the tubules are small, and only Sertoli cells and spermatogonia are demonstrable (NOWAKOWSKI, 1952). In children with AGS testicular development parallels the bone age following the initiation of treatment. True precocious testicular maturation may occur in such cases.

6. Estrogen Therapy. Estrogens in pharmacological doses inhibit the release and production of gonadotropins. The ratio of FSH to LH is not altered. There is first an acute fall in LH, FSH, and testosterone. A steady and slow decline in gonadotropins, testosterone and testicular volume follows. Even after months of therapy, testosterone levels are somewhat higher than in females and gonadotropins lie in the range seen before puberty. After withdrawal of estrogens gonadotropins rise first, followed slowly by a rise of testosterone levels. Physiological doses of estrogens seem to have no effect on gonadotropins. The observation that testosterone levels may be decreased during estrogen treatment despite normal levels of LH – seen especially with TACE – has led to the suggestion of an additional direct inhibitory effect of the estrogens on the testis (BURGER, 1972). Estrogens lead to testicular atrophy, azoospermia, and manifestations of androgen failure and gynecomastia in the man. As used in the treatment of diseases of the prostate, they usually lead to gynecomastia. Sensitivity of the glandular epithelium of the male breast varies widely. Response of the gland can be prevented by X-ray irradiation. Libido decreases, ejaculation becomes impossible, pubic and axillary hair may regress, and the appearance of these patients differs little from that of subjects who have undergone castration late in life. Gonadotropin excretion is no longer measurable, even with relatively low doses of estrogens. The 17-ketosteroids are usually reduced, the testes become smaller, and the histological picture is similar to that of the testes in pituitary failure (see p. 476, Fig. 19a). Testicular damage is reversible after short-term estrogen treatment over some weeks or months. LH deficiency causes atrophy of the Leydig cells.

The same pathogenesis is assumed for *hypogonadism of liver cirrhosis* and of hepatitis. Clearance and inactivation of estrogens may be disturbed due to the liver damage, but increased estrogen production, decreased disposal and elevated levels of binding proteins have also been discussed (BURGER, 1972). This may result in a lower level of unbound testosterone (GALVÃO, 1973). The changes in the testes in liver cirrhosis can be of varying severity. Severe testicular atrophy has also been observed as a concomitant symptom, especially in hemochromatosis. This situation is less clear.

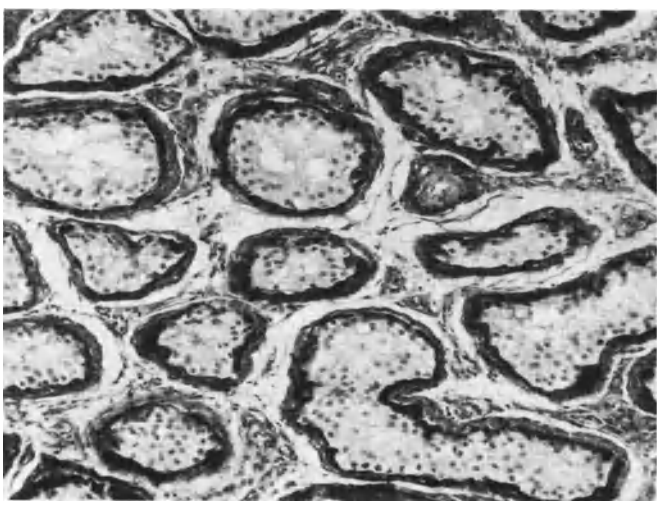


Fig. 16. MB 8819/55, 58-year-old man. Testicular atrophy after treatment with estrogens. Van Gieson, 140:1

Gonadotropin levels have been found to be reduced in some cases and normal or even elevated in others. There is no direct connection between the degree of testicular atrophy and the intensity of the iron deposition in the anterior pituitary lobe. Impaired breakdown of estrogens cannot, however, always be proved in liver cirrhosis. There may also be primary testicular damage due to deposition of iron (GILBERT-DREYFUS, 1967). There is a possibility that the same noxious agents cause liver cirrhosis and damage to the testes.

β) Tubular Insufficiency

It is not yet certain whether isolated FSH failure can also occur in the adult male. This constellation (no FSH, normal adult values for LH) is present in boys with pubertas praecox (KELLER, 1968). These findings have to be proved by immunological gonadotropin determinations.

γ) Interstitial Insufficiency

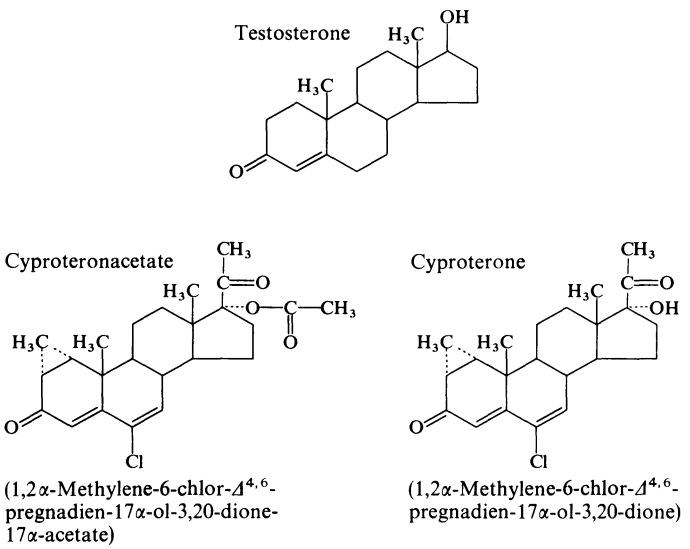
The syndrome of the "fertile eunuch", in which hormone formation is impaired in the presence of normal or almost normal semen production, is due to isolated LH failure (MCCULLAGH, 1953). Subjects with this syndrome have underdeveloped sexual characteristics, almost always with testes of normal size, and normal or slightly disturbed spermatogenesis, but there is hypoplasia or complete absence of Leydig cells. The semen volume is reduced, and the sperm count is normal or slightly diminished due to androgen deficiency. Complete aspermia may occur. FSH excretion is normal, but in contrast, LH excretion has been found to be reduced in a few cases. With improved methods, however, even elevated LH has recently been found. So the syndrome may be one of primary hypogonadism and be caused by damaged Leydig cells. The 17-ketosteroid excretion is in the lower normal limits or is reduced. Secondary sexual characteristics develop under chorionic gonadotropin as well as under testosterone.

5. Anti-Androgens ("Chemical Castration")

Anti-androgens are substances which competitively inhibit or block androgenic effects of other substances and of testosterone at the peripheral receptors.

A large number of steroids which inhibit or abolish androgenic effects have been examined (LERNER, 1964; DORFMANN, 1968; NEUMANN, 1968). However, up to now, the only compounds of theoretical and clinical importance are danazol, an ethinyl testosterone analogue (SHERINS, 1971) and cyproteron and cyproteron acetate. The former is biologically inactive, and the latter has a strong gestagenic effect and inhibits the complex formation between 5α-dihydrotestosterone and the intracellular receptors (FANG, 1969).

The pure anti-androgens, such as cyproteron, block the receptors of the negative feedback system. An uninhibited secretion of the releasing factors and an increased production of gonadotropins results, so that the inhibitory effect on the endorgans may finally be overcome by overproduction of testosterone (NEUMANN, 1971). Cyproteron acetate, however, with its



(1,2α-Methylene-6-chlor-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dione-17α-acetate)

(1,2α-Methylene-6-chlor-$\Delta^{4,6}$-pregnadien-17α-ol-3,20-dione)

Fig. 17. Formulas of testosterone, cyproterone acetate, cyproterone

marked progestational effect, inhibits the release of LH and FSH at the same time and thus has a lasting anti-androgenic effect (NEUMANN, 1970). Thus, cyproteron leads to an increase in LH, whereas cyproteron acetate inhibits both LH and FSH.

Cyproteron given to pregnant animals has an irreversible effect on fetal sexual differentiation. Cyproteron acetate inhibits spermatogenesis and causes histological alterations in the germinal epithelium. These effects are, however, entirely reversible after 3 to 20 months (OTT, 1972). Inhibitory effects normally exerted by androgens are abolished, but the processes induced by androgens are also retarded. The actual formation of the gonads themselves is not influenced, but the Wolffian ducts are suppressed. The Müller's ducts are unaffected, insofar as they are not regulated by factors independent of androgens. In the male fetus the gonaducts and prostate are absent, while a vagina but no uterus is formed. External sexual organs remain feminine since no hormonal effect is necessary for their formation. In the male rat mammary glands are formed as in the female animal, and they can be stimulated by estrogens and progesterone. The picture of testicular feminization is reproduced in the fetus (see p. 726). This supports the hypothesis that peripheral resistance to androgens is the cause of this hereditary disorder. In the adult man castration or the "fertile eunuch" syndrome or the male climacteric is simulated.

Libido and potency decrease, but the secondary sexual characteristics regress only slightly in the adult. Spermatogenesis is greatly impaired, and gonocytes mature only at the stage of the spermatids, whereas the Leydig cells become rather hypertrophied. Growth and function of androgen-dependent glands, such as the prostate, the preputial glands, and particularly the cutaneous sebaceous glands, are inhibited. The hypothalamic-pituitary inhibition of the gonadotropins is abolished by cyproteron even in the presence of exogenous testosterone. Castration cells arise in the anterior pituitary (NEUMANN, 1967). The hypothalamus becomes readjusted to the rhythmic regulation of the ovaries in the hirsute and amenorrheic woman. In the human, as in animals, total excretion of the gonadotropins rises (VOIGT, 1967). Apart from this, cyproteron might also act as a false transmitter in the synthesis and metabolism of testosterone. Changes in the amounts of precursors and metabolites of testosterone have been found (APOSTOLAKIS, 1967; BREUER, 1968). Enzymes stimulated by testosterone, such as LDH and acid phosphatase, decrease. A further central action demonstrated beyond doubt in animal experiments as well as in the human is the decrease in libido.

Cyproteron and cyproteron acetate have already proved clinically satisfactory in psychiatry, when used for excessive sexual drive which has caused subjective suffering to the patient or led to legal conflicts. The advantage of this therapy over estrogen therapy is obvious, the latter leading to gonadotropin inhibition and thus to testicular atrophy and to gynecomastia. Its effect against acne and hirsutism has also been tried in the human. The usage of cyproteron in women of childbearing age carries the danger of a male child being feminized, if a patient is unaware of her pregnancy. Local use against seborrhea and acne might be tried. Use in certain types of amenorrhea, in pubertas praecox in boys, and in the congenital adrogenital syndrome to prevent premature fusion of the epiphyses is under examination. In the proper dosage it could also be considered as a male contraceptive agent without side effects. Carcinoma of the prostate gland would be another valuable indication, if the castration and feminizing effects of estrogens could be thus avoided. However, very high doses are necessary, and the cost is very high.

The daily dose of cyproteron acetate varies between 50 and 150 mg, depending on the indication. The action of 0.5 mg testosterone on the sebaceous glands can be inhibited by 1 mg cyproton acetate. The relation to testosterone in other organs is still unknown. Clinical trial of cyproteron is not contraindicated in any of these patients, with the exception of women of child-bearing age, since it is biologically inert and has no known toxic effects.

Soldactona, an aldosterone antagonist, causes a decreased secretion of testosterone from the testes (DYMLING, 1972). DIMP, a non steroidal antiandrogen, blocks the effect of ketosterone and other androgens on their target organs (BORIS, 1973).

6. Differential Diagnosis of Male Hypogonadism

Panhypopituitarism is of prime importance in the differential diagnosis of male hypogonadism (see p. 93). In addition to eunuchoid signs, in panhypopituitarism there are clinical features of hypothyroidism, which can be best seen from the tired, puffy facial expression (Chap. V, Fig. 3b). In contrast, adrenocortical insufficiency, which is present at the same time, is less apparent apart from pallor of the skin. It is detected from the decreased physical capacity. Thyroid and adrenal function tests differentiate pituitary insufficiency from hypogonadism with certainty.

The constitutional anomaly, "pseudo-eunuchism", can be distinguished without difficulty from true hypogonadism by the normal size of the testes and intact sexual function. The boy-like face, childish voice, poor growth of the beard, and scanty axillary and pubic hair may, however, suggest eunuchism at first. This constitutional anomaly must be due to an inadequate response of the tissues to testosterone.

On the other hand, differential diagnosis between hypogonadotropic hypogonadism in youth and delayed puberty can be difficult. It may sometimes be impossible before the 18th year (see Chap. XIX). Laboratory tests can be useful: in delayed puberty, plasma testosterone is within normal adult levels even before the appearance of secondary sexual characteristics, and it can be stimulated by HCG.

Hormone estimations and testis biopsies are generally necessary for the differential diagnosis of the sub-groups. The following principles sometimes facilitate classification, even without hormone estimations or biopsies.

1. Disorders of the tubular system lead to azoospermia and, in general, to small testes. Masculine sexual characteristics are not affected in isolated tubular failure.

2. In contrast, insufficiency of the Leydig cells can be recognized by the absence of secondary sexual characteristics (beard, moustache, pubic and axillary hair, prostate). The testes need not be reduced in size.

3. In differentiating between primary and secondary insufficiency, gonadotropin estimation can be replaced by the HCG test to estimate the functional reserve of the Leydig cells. Leydig cells respond to chorionic gonadotropin in secondary insufficiency, whereas they are uninfluenced in primary insufficiency. A normal response is indicated by the increase in plasma testosterone, 17-ketosteroid and estrogen excretions, and by the appearance of secondary sexual characteristics.

4. Positive sex chromatin indicates Klinefelter's syndrome.

7. Therapy (for age-dependent indications see Chap. XIX)

There are two main methods of treating male hypogonadism: substitution and stimulation of testicular maturation with chorionic gonadotropin. Only substitution can be considered in primary testicular insufficiency. In contrast, in secondary insufficiency, there is the theoretical possibility of using gonadotropins to stimulate endogenous production of testosterone. The preparation obtained from pregnant mare's serum (PMG), which contains FSH, causes antibodies to be formed in the human, and thus cannot be used therapeutically. Human gonadotropin obtained from the pituitary is available in limited amounts only for research.

Preparations containing predominantly FSH can be gained from urine of menopausal women (human menopausal gonadotropin, HMG). They are still very expensive but are available commercially (Pergonal, Humegon). On the other hand, chorionic gonadotropin extracted from the urine of pregnant women (human chorionic gonadotropin, HCG), is available. The action of this preparation is similar to that of LH. Chorionic gonadotropin is suitable for short courses of treatment but not for long-term treatment, where testosterone is preferable. The indications for chorionic gonadotropin are therefore limited to specially selected cases of hypogonadotropic eunuchism and to cryptorchidism (see p. 469, 477). Re-establishment of spermatogenesis and fertility with HMG and HCG is now theoretically possible in men with no pituitary gland (see p. 485).

Table 5. Synopsis and differential diagnosis between primary and secondary, tubular and interstitial insufficiency

	Total gonadotropin excretion	Spermatozoa	Secondary sexual characteristics 17-ketosteroids	Reaction to chorionic gonadotropin
Primary insufficiency				
Tubular and interstitial	+ +	0	−	0
Tubular	+ + or n	0	n	n or 0
Interstitial	+ +	n or −	n or −	0
Secondary insufficiency				
Tubular and interstitial	− or 0	0	−	n
Tubular	n	−	n	n
Interstitial	n or −	n or −	−	n

n = normal; + = increased; 0 = missing; − = decreased.

a) Substitution

The genuine testicular hormone, testosterone, and its ester are used particularly for substitution therapy. The dosage is adjusted to the severity of the manifestations of failure and to individual response. Estimation of plasma testosterone is expensive for the individual case, but it demonstrates that normal values of plasma testosterone are achieved in the adult man with complete substitution. Initial therapy should be as intensive as possible in cases of complete failure. A dose of 25 mg of testosterone propionate daily or 50 mg 2 to 3 times weekly should be given intramuscularly. The onset of action of this therapy is rapid, and it may be apparent within hours. Erection is possible after a few days. Pubic and axillary hair begin to grow after 1–2 weeks. A change is noted in the voice after 3–6 weeks. As in puberty, acne may develop on the face and trunk. A mild, often painful gynecomastia becomes apparent, such as occurs quite frequently in puberty. The beard and moustache are the last to appear, but they hardly ever attain normal growth. Patients with prepuberal hypogonadism need hardly shave more than twice weekly after successful treatment (Fig. 9). Failure to completely normalize secondary sexual characteristics (JOHNSEN, 1967) is obviously due to the fact that treatment was not begun early enough.

After full development of secondary sexual characteristics in 2–4 months, a maintenance dose of 25 mg testosterone propionate 2 to 3 times weekly is usually sufficient. The use of depot preparations is advantageous. Subcutaneous implantation of pellets of free testosterone is no longer used.

It is now convenient to give a monthly injection of the slowly dissolvable ester or ester compound, such as undecylenate or valerianate, caprinoylacetate, enanthate. Numerous commercial preparations combine rapid- and prolonged-action testosterone esters. They are given intramuscularly in an oily solution in a dose of 250 mg (150–300) once a month or once every three weeks in order to compensate complete testicular failure adequately. Higher long-term doses are only indicated in the treatment of cancer, to obtain anabolic effects, or to prevent abnormal height. Overdosage must be avoided, as it can lead to too frequent ejaculations, to priapism, or to polycathemia.

These ester compounds can be used right from the start of the substitution therapy since their onset of action is rapid. They must, however, then be given in higher doses, e.g. 250–500 mg once a fortnight. The effect of testosterone, however, is variable according to the disease, a fact that has to be considered. In particular, the sensitivity to testosterone is diminished in growth-hormone deficiency (ZACHMANN, 1969).

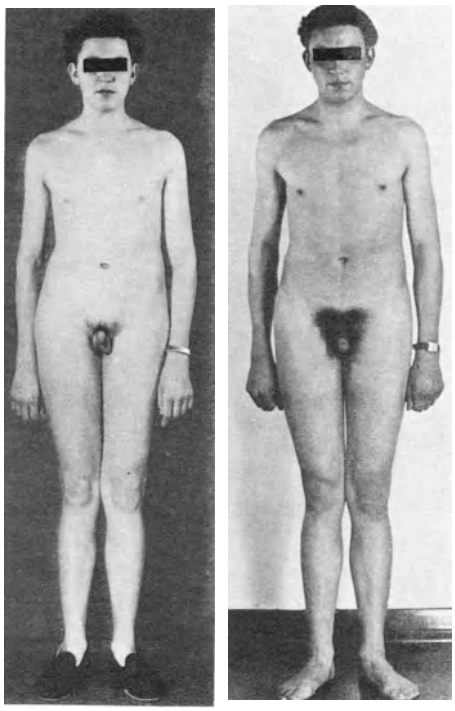
Smaller doses are adequate for the treatment of the male climacteric. Doses of 10–25 mg testosterone propionate can be given intramuscularly twice weekly, or 50 mg of a depot preparation every 4 weeks.

Oral testosterone therapy has the advantage of reducing the patient's dependence on the doctor, but it has the drawback of being less effective, more costly, and of involving the risk of cholestatic icterus. The methyl derivate of testosterone is used for oral administration. It is thought to reach the circulation by passing along the lymphatics and bypassing the portal circulation. Daily doses of 25 – 150 mg are necessary for replacement treatment. A halogenated testosterone derivative, fluoxymesterone, is a more effective oral preparation. A dosage of 5–20 mg daily permits appropriate substitution, which is not, however, equivalent to that achieved with preparations for injection. Mesterolone, 1α-methyl-5α-androstane-17β-ol-30, can be used orally withouth the risk of biliary stasis, since is does not possess a methyl group at atom 17. It is not converted into estrogens. The preparation has undergone extensive clinical trial, and it appeared that the effect in the human does not differ from that in animals, i.e. that effective doses will not inhibit gonadotropins (PETRY, 1968; LUDVIK, 1970).

Testosterone, like numerous other steroids, has a slight sodium-retaining effect. Edema may therefore arise with high doses, especially in elderly patients. This can be prevented by giving a low-sodium diet. See p. 455 for the effect of testosterone on spermatogenesis.

b) Stimulation of Hormone Production

In isolated gonadotropin failure, a trial with chorionic gonadotropin (HCG) might be indicated to promote maturation of the testes and hormone production. Substitution with testosterone must be continued for the rest of the patient's life. This treatment can prevent the absence of secondary sexual characteristics and metabolic disorders as well as psychic effects, but the patients remain sterile. HCG, on the other hand, can bring about maturation of the testes. They become larger and Leydig cells develop. HMG is needed in addition to produce spermatogenesis. HCG must be injected in high doses over a long period of time, at least 3 months. Many failures are due to inappropriate dosage. In our experience, a weekly or twice



a b

Fig. 18a and b. 23-year-old. Idiopathic eunuchoidism. a) Before treatment. b) 5 months after the first treatment with chorionic gonadotropin (120000 IU in 3 months)

weekly injection of 5000 units is very effective. Others advise 300–2500 units daily.

The testes respond rapidly to this therapy. The first signs of development are visible after only one week, and the whole process to complete development takes place within 3 months, in comparison to the 3–4 years needed for the physiological course (Fig. 18). Repeated testicular biopsies during treatment with chorionic gonadotropin show rapid growth of Leydig cells at first, followed by hypertrophy. At the same time, the tubular tunica propria thickens, which, however, does not signify degeneration but is merely an effect of testosterone (BARTTER, 1952). Sertoli cells mature, a lumen develops in the tubules, and the cells show signs of spermatogenesis (Fig. 19). Semen with numerous spermatozoa can be attained after 3 months of treatment even in a case of complete tubular immaturity. Plasma testosterone, 17-ketosteroid, and estrogen levels rise and may reach normal or higher values in a short time.

Initial treatment lasting for 3–6 months is followed by a pause of 3–6 months. As discussed in the description of hypogonadotropic eunuchism, success will be lasting in some patients, in whom the disorder is due only to failure of the normal mechanism to release puberty. In other patients, secondary sexual characteristics will

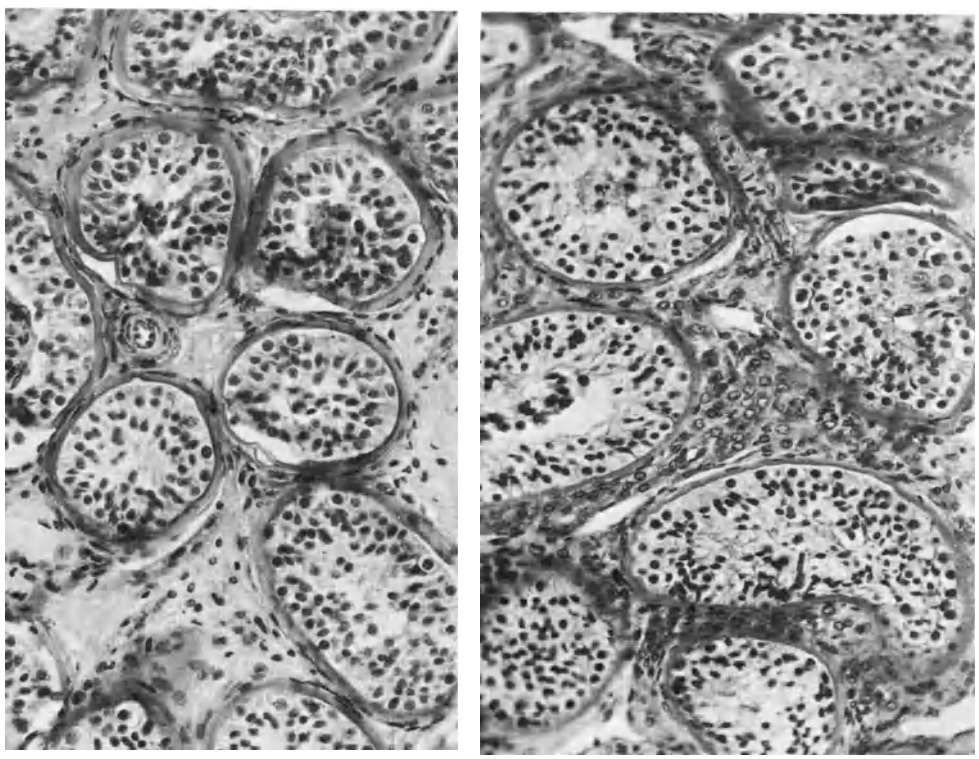


Fig. 19a and b. MB 268/53 and 4837/53, 23-year-old man. Testis in hypogonadotropic hypogonadism. a) Before treatment. b) After treatment with gonadotropins. Tubules somewhat better developed. Tunica propria narrower. Foci of Leydig cells. HE, 180:1

disappear gradually over the course of 2–5 months. Often a higher degree of maturation is retained after treatment. In these cases, a second and third course of HCG is indicated. The best possible degree of maturation is achieved after the third course, and any disturbing androgen deficiency remaining after this must be compensated with long-term testosterone substitution therapy. According to BARTTER, cases with a more mature histological picture have more hope of lasting success than the completely immature forms.

The course of HCG injections is tedious for both patient and doctor, and although a partial lasting success may be attained, life-long substitution with testosterone cannot usually be avoided. It is therefore often preferable to start with testosterone substitution, even in cases of secondary hypogonadism. Testosterone substitution can be withdrawn at least for a limited period when fertility is to be achieved. HCG, later combined with HMG, can then be given (see p. 485).

F. Cryptorchidism (Undescended Testes)

A. PRADER

With Contributions by
CHR. HEDINGER

1. Definition and Incidence

In German-speaking countries, the term cryptorchidism is used only for testes retained in the abdomen. In this chapter, however, as in Anglo-Saxon terminology, *cryptorchidism* or *undescended testes* generally refers to the incompletely descended testes. In contrast to this, the term "*ectopic testis*" is used when the testis lies in some position which never occurs under normal conditions. A normal variety is the testis lying partly inguinally and partly within the scrotum. This variety is still incorrectly looked upon as cryptorchidism (migratory testis, pendulous testis).

Descent normally occurs during the fetal peroid and is usually complete by the time of birth. The factors responsible for descent have not been fully identified. Chorionic gonadotropin probably plays a significant role.

The incidence of cryptorchidism is 2–3% in newborns born at full term (SCORER, 1964; VILLUMSEN, 1966), and higher in premature infants. Delayed descent commonly occurs during the first few weeks or months of life, so that the incidence is merely 0.8% at school age (COUR-PALAIS, 1966). Higher incidences are due to poor examination techniques (COUR-PALAIS, 1966). Contrary to previous assumptions, spontaneous descent appears to occur only during the first few months of life and occasionally during puberty. The figures for the incidence in adults vary between 0.2 and 0.9%. Cryptorchidism is bilateral in 10–20% of all cases. Ectopia is about ten times less common than retention.

2. Etiology

An endocrine disorder, anatomical obstruction, and inferior testicular anlage are etiological factors to be considered. Occasionally, it is a simple hereditary characteristic due to an irregular dominant inheritance (KLEIN, 1963).

Nothing definite is known about the *endocrine causes* of cryptorchidism. There is no doubt that descent is promoted during fetal life by adequate amounts of chorionic gonadotropin and by the development of hypophyseal gonadotropins during puberty. However, it is not known whether cryptorchidism can be related to a gonadotropin deficiency during the fetal period. In most cases of cryptorchidism no endocrine disorder apart from secondary gonadal atrophy can be demonstrated. Cryptorchidism is a common but not obligatory feature of hypogonadism (p. 464), pituitary dwarfism (p. 98), and some types of male pseudohermaphroditism (see Chap. XII), but it is not known whether the cryptorchidism is caused by the pituitary or gonadal deficiency or by the disorder underlying the pseudohermaphroditism.

Anatomical obstructions can lead to ectopia as well as to cryptorchidism. The inguinal canal is not patent enough, or the spermatic artery is short, or a congenital hernia is present (persistence of the processus vaginalis). The hernia prevents descent or results in adhesions and fixation of the testis in the wrong position. An anatomical obstruction causing cryptorchidism can only be revealed by surgery in the majority of cases.

It appears that about 50% of testes still undescended after puberty were already inferior in the anlage stage. In other words, *testicular dysplasia* or *testicular hypoplasia* is present. It is, however, only possible to prove this by histological examination. In one third of cases of unilateral cryptorchidism, both testes are primarily abnormal (HECKER, 1967). In isolated cases it is one symptom among multiple malformations or a symptom of a dysmorphic syndrome (male Turner's syndrome, Prader-Labhart-Willi syndrome, persistent oviduct, etc.).

3. Consequences of Cryptorchidism

The consequences of cryptorchidism are multiple. Testes situated in the inguinal canal are much more prone to *traumatic* injuries than scrotal testes. In addition, *testicular torsion* is thought to be more common. Pains are not infrequent in the region of the undescended testis, and an *incarcerated hernia* and *testicular torsion* must be considered in such cases. During adulthood, but not during childhood, *malignant tumors* are more common in undescended testes (10 to 50 times more common, depending on the author) than in scrotal testes. However, even after an undescended testis has been transferred into the scrotum, it appears to have a predisposition to developing tumors (GILBERT, 1941). Thus, it would appear that not the maldescent but rather the testicular hypoplasia or atrophy must be considered the cause of the increased frequency of tumors.

The *psychological complications* of cryptorchidism have also been stressed by various authors. There is no doubt that disadvantageous psychological reactions arise in boys and men with cryptorchidism, who are aware of "inadequately developed manhood".

Testicular atrophy is by far the most important consequence of cryptorchidism. This is apparently due to the fact that the temperature of the surroundings of undescended testes is 1.5–4° C higher than in the scrotum. Tubular development and spermatogenesis are very much affected by this, whereas development of the Leydig cells and their endocrine function are little influenced.

4. Morbid Anatomy

CHR. HEDINGER

Undescended testes are usually underdeveloped and are definitely reduced in size in the adult. The microscopic picture is determined primarily by the age of the patient but there is no proven relationship to the degree of alterations of retention, i.e. to the localization of the retained testis. No pathologic processes can be detected immediately after birth unless testicular dysplasia is present. However, pronounced changes arise during the first months and years of life, appearing mainly as a marked reduction in the number of spermatogonia and a usually rather discrete decrease in the diameter of the tubules compared with normally situated testes. Because of this, tubules devoid of spermatogonia are not uncommon even during the first few years of life (SALLE, 1968). As far as this question can be answered at present, in the majority of cases it appears that this is an acquired and not a congenital disorder (HEDINGER, 1971). All these changes become even more marked with the onset of puberty. The tubules continue to develop but remain smaller than normal. The tunica propria is thickened and hyalinized, Sertoli cells mature but the germinal epithelium is usually already absent or undergoes degeneration. The histological picture of the undescended testis in the adult is thus characterized by the combination of incomplete tubular development with simultaneous tubular atrophy advancing to complete tubular sclerosis. In contrast, the Leydig cells are usually well maintained, and are not uncommonly densely packed with increased amounts of lipids, which lend a peculiar golden yellow color to the cut surface of the testis on macroscopic examination. In about half the cases, so-called hypoplastic zones are recognizable (Fig. 20). They are composed of proliferations of peculiar tubules which contain only undifferentiated epithelia and no spermatogonia. These zones look similar to adenomas and may be mistaken for Pick's tubular adenoma, which also occurs particularly often in undescended testes. Concretions or so-called ring tubules appear to be somewhat more common in undescended testes than in normally situated testes (SCHEIBLI, 1968; HUBER, 1968).

5. Incretory and Secretory Function of Undescended Testes

Clinically, no differentiation is usually possible between primary hypoplastic testes and secondary atrophic testes. In both forms the testes are unobtrusive before puberty and strikingly small after puberty. Spermatogenesis is equally impaired in both forms. No mature sperms or only a few can be found in the ejaculate, and the patient is usually sterile. This is understandable in bilateral cryptorchidism, but surprisingly, the same features are often found in cases of unilateral cryptorchidism. In such cases, presumably bilateral testicular hypoplasia is present and the testes are not atrophic. In other cases, compensatory enlargement of the healthy testis can be observed (LARON, 1969).

In contrast to spermatogenesis, androgen production is not reduced or only slightly reduced. Secondary sexual characteristics are fairly to very well developed. Excretion of 17-ketosteroids and testosterone is normal or slightly reduced and is not as strongly stimulated by chorionic gonadotropin as in healthy subjects. Gonadotropin excretion is usually elevated due to tubular insufficiency. Thus, primary hypergonadotropic hypogonadism arises with

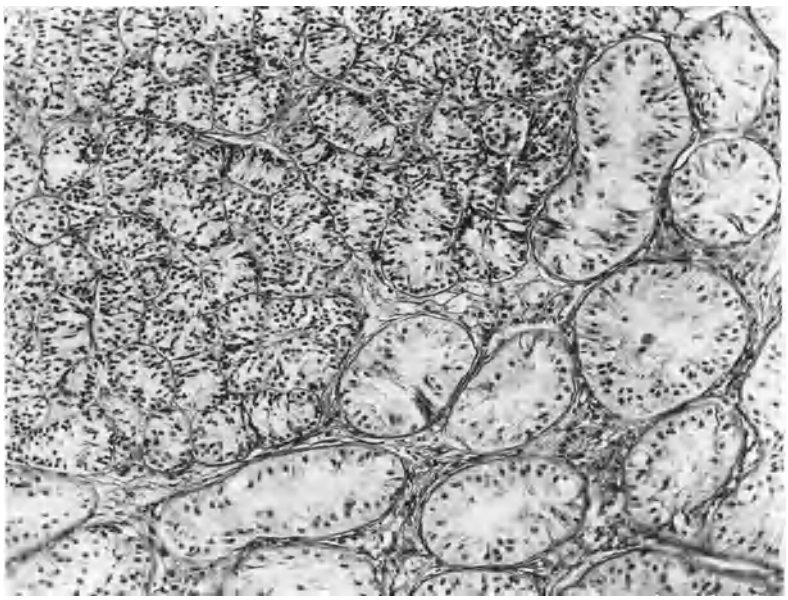


Fig. 20. MB 8853/51, 24-year-old man. Cryptorchidism with defined hypoplastic zone (upper left half of the picture reminiscent of a tubular adenoma). In the rest of the testicular tissue atropic tubules which are lined almost exclusively with Sertoli cells. Leydig cells recognizable in small groups. HE, 140:1.

secretory insufficiency and incretory function is just about adequate (p. 463). *In-vitro* experiments suggest some degree of 3β-dehydrogenase deficiency in the biosynthesis of testosterone (HAMILTON, 1970).

6. Examination Technique and Classification

There are numerous cases where a diagnosis of cryptorchidism has been wrongly made and the patient unnecessarily treated for this condition due to poor examination technique. Careful and correct examination is therefore of greatest importance. The patient should be examined in the standing and lying positions. The examination room must be warm since a cold stimulus promotes retraction of the testes. Bimanual examination is most suitable. One hand squeezes the inguinal canal and the subcutaneous pocket lying between the inguinal canal and scrotum against the other hand palpating away from the scrotum. If no testes are palpated at first, success can occasionally be achieved by making the patient cough or shout or by examining him in a warm bath.

Palpation findings permit a certain degree of diagnostic differentiation and classification into the various forms of cryptorchidism. The primary factor is to determine whether testes are palpable or not.

If the testes are not detectable by careful examination, they are lying in the inguinal canal (canalicular inguinal retention), or in the retroperitoneal space (abdominal retention), or are completely absent (anorchia). Anorchia can be recognized by the failure of development of secondary sexual characteristics, by the lack of response of the excretions of 17-ketosteroids and testosterone to stimulation with chorionic gonadotropin, and by failure to find the testes on surgical exploration although the ductus deferens is present. Among 748 children admitted to the surgical wards of the Children's Hospital in Zurich and operated on for cryptorchidism, bilateral anorchia was confirmed in 6 cases and unilateral anorchia in nine cases on the basis of the ductus deferens ending freely.

Palpable testes are practically always situated subcutaneously. Testes palpable in the inguinal region which can be easily pulled into the scrotum are a normal variety, as has already been mentioned (*migratory or pendulous testes*), and must not be looked upon as cryptorchidism. Very often, testes can just barely be pulled into the scrotum and rebound at once when released (retractile testes). This is the mildest form of cryptorchidism; it has a good spontaneous prognosis, and usually responds immediately to chorionic gonadotropin.

Testes palpable in the inguinal region (inguinal testes, inguinal retention) are less mobile, and an anatomical obstruction or inguinal ectopia cannot be excluded. The other forms of ectopia (ectopia femoralis, perinealis, penilis) are easily recognized from the abnormal position of the testes.

7. Treatment

There are only two therapeutic possibilities: treatment with chorionic gonadotropin and surgery (orchidopexy). Whereas it is unanimously agreed that the onset of puberty is the latest point for starting treatment, since testicular atrophy is otherwise inevitable, opinions differ as to the optimal age to start treatment. The fact that the tubular diameter and sperm count are lower than normal even in the 5th or 6th year (HECKER, 1967; SALLE, 1968) has led to the recommendation that treatment be started as early as possible. The question cannot be answered definitely since it is not clear at present whether these changes are due to the abnormal site of the testes or are a primary disturbance, and assuming there is a secondary disorder due to the abnormal position, whether this secondary disorder is reversible and if so, for how long. We therefore recommend starting treatment with chorionic gonadotropin as early as possible, i.e. from the 5th year, adjusting the doses to produce increased erections and mild penis enlargement but no pubic hair if possible. This side effect disappears after treatment is stopped. Between the ages of 5 and 10 years, 6–8 injections of 1500 U are given in 3–4 weeks; between the 10th and 13th years, 10 injections of 1500 U in five weeks or 3 injections of 5000 U in 3 weeks. Urinary excretion of testosterone rises significantly. Absence of the clinical reaction mentioned above and failure of testosterone to rise confirm the presence of true anorchia. The degree of success cannot be predicted in the individual case and is presumably dependent on the unknown causes of cryptorchidism. Success is achieved in 90 to 100% of cases of retractile testes, and the therapeutic indication is questionable here. Success is obtained in about 40% of cases of inguinal retention and in 20–30% of cases of abdominal retention (BIERICH, 1967; KNORR, 1970). Results are somewhat poorer in unilateral than in bilateral cryptorchidism. It may be assumed that in successful cases the testes would have descended spontaneously later on in puberty under the influence of endogenous gonadotropins, but there is some fear that this delay affects later fertitity. This form of treatment involves no risk. Descent of the inguinal and abdominal testes does not ensure fertility. The testes often remain smaller than normal, and normal spermatogenesis is probably only possible in half the cases at most. Failure to respond is an indication for surgery. Hardly any result is to be expected from the second course of chorionic gonadotropin.

In contrast to treatment with gonadotropin, orchidopexy is almost always successful as far as the position of the testes is concerned. It is the only form of treatment in the presence of anatomical obstruction, incarcerated hernia, testicular torsion and ectopia. Surgery results in just as little functional success as gonadotropin treatment in the presence of testicular hypoplasia. By and large, surgery using suitable methods can be described as harmless. However, subsequent testicular atrophy is almost inevitable if the testis can only be transferred to the scrotum under tension due to short vessels or an unsuitable surgical method. For reasons already discussed, the testes often remain abnormally small after the operation. Nor are the chances of fertility very good, particularly when the case is referred to the surgeon after failure to respond to chorionic gonadotropin. In summary, there is still a considerable amount of uncertainty as to the optimal treatment of cryptorchidism with the present-day state of knowledge. For the time being, the following principles for treatment of uni- and bilateral cryptorchidism in endocrinologically healthy boys can be recommended:

1. First exclude the migratory or pendulous testes which can be mistaken for true cryptorchidism. Migratory testes, in contrast to undescended testes, can easily be manipulated into the scrotum and require no treatment.

2. Every case of ectopic testes diagnosed clinically (position outside the normal path of descent) and of cryptorchidism with a manifest hernia should be operated on during babyhood or early infancy.

3. Treatment is urgently indicated in uncomplicated cases of undescended testes, and should begin as soon as possible after the 5th year of life and definitely before the onset of puberty.

4. If treatment with chorionic gonadotropin is decided upon, then a course of injections of 1500–5000 U over three to five weeks, making a total dose of 9000–15000 U, can be recommended.

5. If surgical treatment is decided upon because of failure to respond to treatment with gonadotropin or because of anatomical complications, careful orchidolysis and funicolysis with subsequent orchidopexy by fixation in the scrotum itself (between tunica dartos and scrotal skin or penetration of the septum) is indicated. Temporary fixation to the thigh gives poor results.

6. Retractile testes, which can just be pulled into the scrotum and recoil immediately, are an indication for treatment. Gonadotropin treatment is nearly always successful.

7. Chances of success with gonadotropins are lower but still fairly good in cases of testes retained in the inguinal region.

8. If the testes are not palpable, therapeutic possibilities are limited. Orchidopexy of abdominal testes can often be performed without difficulty, but occasionally only with great tension on the vessels, with consequent testicular atrophy. A previous attempt at treatment with gonadotropin is thus advisable, especially since even partial succes improves the prognosis of subsequent orchidopexy. A pronounced rise in 17-ketosteroid and testosterone levels during this treatment indicates the presence of testes, whereas absence of this rise suggests anorchia.

Treatment of cryptorchidism in adulthood also presents a difficult problem. Normal spermatogenesis cannot be achieved either with chorionic gonadotropin or by orchidopexy. The danger of increased predisposition to tumors must also be considered in this age group. Certain authors recommended castration for this reason, but then loss of function of the Leydig cells must be taken into account and compensated by substitution with testosterone.

G. Sterility

The incidence of sterile marriages has been estimated at 10%. In 30–50% of cases, the cause is found to lie with the man. The figures have remained more or less the same for the past three generations, and sterility affects all social groups to the same extent. There are different grades of fertility. The marriage will be fertile when a person of inferior fertility combines with a very fertile partner, whereas the combination of two partners whose fertility is low will remain childless. It is probable, but also difficult to prove, that inferior or incompatible sperms are the cause of frequent abortions.

1. Etiology and Pathogenesis

a) Functional and Anatomical Disorders of the External Genital Organs

History and clinical examination will quickly disclose impotence, hypospadia, and other anatomical abnormalities which will impede the normal course of coitus.

b) Urological Causes

Obstruction of the spermatic ducts is responsible for 25% of cases of azoospermia in the presence of normally large testes. The condition most often arises after gonococcal or tuberculous epididymitis. It is important to recognize the obstruction, since some of these cases are suitable for correction. The testes biopsy is therefore necessary in a case of azoospermia with normally large testes. It usually shows a normal picture in obstruction of the spermatic ducts, but spermatogenesis is slightly diminished in 20% of cases. The success rate for latero-lateral vasoepididymostomy performed by an experienced surgeon is variously quoted as 20 to 70%. Although the percentage of curable cases of azoospermia with normally large testes is relatively small, it is still worth trying. The patients should be informed in advance about the low chance of success.

Oligospermia or reduced motility of the sperms is infrequently due to changes in the spermatic and urinary tracts and in their appendage organs (prostatitis, vesiculitis).

"Pseudo-oligospermia praecedens" denotes impaired mobilization of the spermatozoa. In this condition, only a few spermatozoa are obtained from the first ejaculation, whereas ejaculation after 2 hours yields a normal number of spermatozoa.

Azoospermia may occasionally be due to retrograde ejaculation into the bladder.

Finally, congenital malformations of the spermatic ducts may cause azoospermia in the presence of testes of normal size (failure of the epididymal head and body to communicate, cystadenomas of the epididymis).

The spermatic ducts and the epididymis are nearly always absent or have malformations in mucoviscidosis. There is nearly always azoospermia (KAPLAN, 1968; VALMAN, 1969) (see p. 462).

Cases of loss of the seminal fluid, aspermia ("aspermatism"), in the presence of otherwise normal sexual function have been reported after treatment of hypertension with the sympathetic inhibitor, guanethidine. Secretions of the prostate and seminal vesicles appear to be inhibited.

c) Impaired Spermatogenesis

See p. 462 for spermatogenic disturbances in hypogonadism. All disorders of the tubular apparatus are associated with azoospermia or oligospermia. Isolated disorders in formation and maturation of the germ cells have been termed as hypospermatogenesis or as spermatogenic arrest (see p. 465). There is usually no certain morphological correlation in the testis biopsy with oligospermia, however, and the etiology of azoospermia or oligospermia re-

mains unknown in the majority of cases despite all the modern investigations. Chromosomal aberrations are responsible for 7.5% of cases of male sterility. They are recognized only by the karyogram and are mostly XY/XXY, XO/XY or other mosaics (KJESSLER, 1966).

Auto-immunological processes may be involved. Autologous and homologous immunization to testicular tissue have been used to produce cellular and weak circulating antibodies, clumping and desquamation of the germinal epithelium and vacuolar degeneration of the Sertoli cells in animal and man before orchidectomy for carcinoma of the prostate (MANCINI, 1965). The phenomenon of spermatozoal agglutination can be found in sterile men, sometimes after contusion of the testes. Antisperma-antibodies in the serum can also be demonstrated in some cases (FJÄLLBRANT, 1965). Antigens can be found in the acrosome of the head of the spermatozoon. They can arise from the accessory organs and can enclose the spermatozoa. These antigens can evoke production of antibodies bound to the vaginal cells and uterus in the woman and thus cause sterility. The antibodies may disappear and the woman become fertile again after a year of abstinence or coitus condomatus.

d) Sterility with Normospermia (So-Called "Biological Sterility")

α) Immunological Causes, Incompatibility of the AB0 Blood Groups
(BEHRMAN, 1960; MACHER, 1964)

Natural, not acquired anti-A and anti-B antibodies (agglutinins) have been found in the cervical mucus of women of group 0. It is assumed that spermatozoa with the antigens A and B can be immobilized by antibodies in the cervical mucus. Direct agglutination has, however, not yet been demonstrated. Some men in the A and B blood groups excrete the A and B substances in the saliva and semen, and it is possible that these substances neutralize the anti-A or anti-B in the cervical mucus. It is also possible that this immunological A-B-0 incompatibility is in some way involved in the diminished fertility of A-B-0 married couples, since incompatible blood group constellations were found more frequently among "biologically" sterile couples than in a control group. Therapeutic measures to overcome this incompatibility are still at the speculative stage (homologous intra-uterine artificial insemination, neutralization of antibodies in the cervical mucus). Antibodies against sperms can also be acquired.

Agglutination of Sperms. The sperms of a small percentage of sterile men agglutinate before or after ejaculation. High titers of spermatozoa agglutinins can be detected in these cases, suggesting autoimmunological processes (RÜMKE, 1965).

β) Nucleic Acid Deficiency of the Spermatozoa

Nucleinic acid in the head of the spermatozoon can be quantitatively estimated by ultraviolet or Feulgen microspectrophotometry. A decrease in the nucleinic acid content in the heads of the sperms has been successfully demonstrated in a few cases of sterility with normospermia. Connections between this and habitual abortion are currently being discussed (LEUCHTENBERGER, 1957; MEYHÖFER, 1963; JOEL, 1962).

Finally, a morphologically, chemically, and immunologically normal semen may also be inferior.

γ) Capacitation and Decapacitation
(see also Chap. X, p. 623)

Capacitation is a process which enables the sperm to invade the ovum inside the uterus, passing through the matrix of the cumulus oophorus and the zona pellucida (AUSTIN, 1970). Capacitation and decapacitation are not yet completely understood. The sperms seem to be coated with a protective layer, the decapacitation factor, while passing along the seminal ducts (CHANG, 1970). This factor is dissolved inside the uterus near the ovum. It has been isolated to a fairly high degree (WILLIAMS, 1970). Capacitated and decapacitated sperms cannot be differentiated, either by light or by electron microscopy. The outer membrane of the head of capacitated sperms is, however, dissolved in the neighborhood of the ovum, and the enzyme-containing acrosome fuses with the ovum, forming a vesicle (BEDFORD, 1970). Male and female hormones have some influence on these processes. Estrogens, LH and HCG seem to promote capacitation while progesterone inhibits it. Capacitation and decapacitation have been proven in many mammals, including the human. Recently, indirect methods, as tetracycline incubation, to investigate the first phase of capacitation, have been developed (DUKELOW, 1971).

2. Diagnosis

When the history is taken, special attention must be paid to the following: frequency of sexual intercourse, number of siblings, number of their children, previous illnesses, particularly

mumps, tuberculosis, venereal diseases, malnutrition, cryptorchidism and the time of descent, age at onset of puberty, indulgence in alcohol and tobacco, mental stress, professional overexertion, exposure to heat, type of dress, particularly whether close-fitting underpants are worn.

Signs of hypo- or hyperthyroidism, diabetes mellitus, vascular diseases and neurological disorders must be looked for in the general examination. Secondary sexual characteristics must be investigated.

Careful examination of the male genitalia is most important. The size of the testes must be noted. This is best measured with the orchidometer giving the volume of the testis (see p. 1036). The epididymis and vas deferens must be palpated, and a rectal examination performed to examine the prostate and seminal vesicles. Attention must be paid to varicoceles, open hernial orifices, and to the possibility of the testes sliding back into the inguinal canal.

Examination of the semen is the first and most important special test. For the methods see p. 493. Any doctor can come to the simple conclusion of whether the semen contains actively motile spermatozoa, but the differential morphological assessment needs special experience.

Normal semen after 3–5 days of abstinence contains 40–250 million sperms/ml, at least 60% of which must be motile, and 60% normally formed (McLeod, 1965). The sperm count falls if the period of abstinence is shorter, and motility is reduced if it is longer. Fertility is reduced when the sperm count is below 40 million/ml (oligospermia). The possibility of fertility is very slight if the count is less than 20 million/ml. The fall in the sperm count is usually accompanied by impaired motility and morphology. Sterility, however, can only be assumed to exist when spermatozoa are completely absent (azoospermia). If conditions are unfavorable in the woman, a high sperm count is needed for conception to occur. Aspermia is described as the inability to ejaculate semen. We use the nomenclature recommended by the Commitee for Male Infertility in Amsterdam in 1959. In German-speaking countries azoospermia is usually used for the absence of spermatozoa in the presence of cells of spermiogenesis; aspermia is applied to the absence of both, and aspermatism indicates the inability to produce semen. Other terms, such as cryptospermia and astheno-teratospermia are unnecessary and are best avoided. Necrospermia indicates that the sperms are immotile and not revivable. Fertility is also thought to be reduced with sperm counts over 250 million/ml.

Table 6. Examination of the male

A. *History*
1. General: venerea, tuberculosis, mumps-induced orchitis, testicular torsion, mechanical trauma of the genitals, genital surgery: orchidopexia, operation of hydroceles or varicoceles, hernia inguinalis operations. Exogenous damage: alcohol, nicotine, heat, X-rays.
2. Genital: coital frequency, timing and technique, ejaculatio praecox (duration of coitus), possibility of impotence.
3. Psychological: wish for children? Reason for wanting children, attitude to wife, professional aims and interests.

B. *Physical examination*
1. General: state of health, blood pressure, sedimentation rate, red and white blood count and blood smear, serological reactions for lues, urinalysis.
2. Genital: cryptorchidism, hypospadia, genital hypoplasia, orchidometry (testicular size), varicocele, hydrocele, inflammation

C. *Sperm examination*
Quantity and quality (see p. 493)

In case of pathological sperm:

D. 1. Endocrinological examination: gonadotropins, 17-ketosteroids and 17-hydroxysteroids, plasma or urinary testosterone, thyroxine, basal metabolic rate and serum cholesterol.
2. Examination of prostatic secretion (massage of the prostate gland).
3. Testicular biopsy, examination of the clear passage of the sperm ducts, rinsing, vesiculography, urethrography).
4. Sex chromatin or karyogram in case of abnormalities of the external genitalia, underdevelopment of the secondary sexual characteristics, small testicles with azoospermia.

It is not certain how much significance can be attached to biochemical investigations in the practical assessment of fertility. The content of fructose (normal 1200–4500 µg/ml) and that of citric acid (normal 30–420 mg/100 ml) in the semen are dependent on the production of testosterone by the Leydig cells. Fructolysis (fructose consumption in 5 hours) and hyaluronidase activity are parallel to the number of motile sperms.

The testis biopsy is indispensible for an exact diagnosis in hypogonadism and in azoospermia. It is sometimes justified for therapeutic reasons, although only a small percentage of hypogonadal men have any hope of their sterility being cured.

On the other hand, the use and indication of the testis biopsy in oligospermia is disputable. No therapeutic conclusions can be derived from it; however, the chance of success can be better assessed from the biopsy and can spare married couples years of disappointing waiting. In cases where the secondary sexual charac-

teristics are present it can definitely exclude an idiopathic eunuchism. In cases where the testes are particularly small (less than 10 cm^3), fertility is almost impossible, and patients can be spared this tiresome investigation.

It is possible that although the testis biopsy is of no use in some cases, all patients in this group can profit from it since it can provide a basis for future therapy.

The so-called Sims or Huhner's test involves microscopical examination of sperm taken from the cervix post coitum. This test is supposed to indicate individual incompatibility between the sperm and the cervical mucus.

3. Therapy

We still have no certain means of improving the quality and number of spermatozoa except in hypogonadotropic hypogonadism. All reports of success with hormones and vitamins apply to subfertile patients with sperm counts between 20–40 million/ml, which would have allowed conception even without treatment. Therapeutic successes of this kind can be fallacious. The hopes of improving oligospermia with HMG outside of pituitary insufficiency have not materialized (McLeod, 1967).

In Zurich we have never observed any improvement of the semen quality during treatment with HMG, 500 U every day and HCG, 5000 U weekly for 3 months in selected patients with a sperm count of less than 20 million/ml and spermatogonia in the testicular biopsy. Other series have shown comparable results (Polishuk, 1967; Mroueh, 1967).

Azoospermia in the presence of normal-sized testes can be due to obstruction of the spermatic ducts. The testis biopsy shows normal histological findings or only slightly reduced spermatogenesis. Patency of the vas deferens is best tested by lavage with lukewarm physiological saline. Vasography with X-ray contrast medium is thought to cause epithelial lesions.

Biopsy from testes of normal size, however, also reveals disorders of information or maturation, such as spermatogenic arrest, hypospermatogenesis, or germinal aplasia. Treatment is hopeless in all three conditions.

The diagnosis of oligospermia can only be made by repeatedly finding lowered sperm counts. Temporary reductions in the sperm count are not uncommon. In oligospermia without signs of hypogonadism, urological causes such as prostatitis must be investigated and cured. Inflammatory components in the secretory product can impair motility of the sperms and thus their fertility. The varicocele may cause sterility (see p. 464) (Tulloch, 1955; Scott, 1962). Operative treatment with ligation of the vena spermatica can improve the quality of the semen in 60–70% (McLeod, 1967). Improvement of the motility was the most constant result. Although 37% of the patients in one series had sperm counts of less than 20 million/ml, pregnancies were achieved in 30–50% (McLeod, 1967). Similar observations were recorded in patients with hydrocele. Spermiogenesis may be impaired either by changes in temperature or by hypoxia (Brown, 1967).

It is doubtful whether advice to limit sexual intercourse to the fertile days of the woman's cycle is of any value. It is true that the sperm count increases with sustained abstinence, but motility decreases at the same time. The chances with frequent intercourse are better than with infrequent intercourse (McLeod, 1967). General advice usually given is the following: The patient should relax, have sufficient sleep, avoid becoming overweight, and have some physical exercise. Close-fitting underwear is thought to be unfavorable since it increases the temperature in the testes.

Table 7. Treatment of male sterility

Cause	Sperm count	Size of testes	Testis biopsy	Therapy
1. Azoospermia in the presence of obstructed spermatic ducts	0	Normal	Normal or slightly decreased spermatogenesis	Surgical
2. Hypogonadism	Decreased or 0	Smaller	Pathologic	HCG, HMG in selected cases
3. Oligospermia	Decreased	Normal	Normal or slightly pathologic	Homologous artificial insemination in selected cases
4. Unknown	Normal	Normal	Normal	None

Postcoital loss of semen can be prevented by the woman's adoption of a suitable position with the thighs drawn up and apart.

When these measures are unsuccessful, mechanical means of concentrating the semen can be considered. Homologous artifical insemination, i.e. insemination with the husband's semen, makes it possible to place semen in the concentrated form in the cervix or in the uterine cavity. The first half of the ejaculate usually contains the majority of the more motile sperms. This fact can be exploited by using a "fractionated ejaculate" for artificial insemination. The semen is obtained by masturbation and is collected in sterile containers. It can be concentrated by centrifugation. Semen from repeated masturbations is collected and stored in the deep freeze. It is then allowed to thaw out and is centrifuged. The semen thus obtained for homologous artificial insemination has a normal sperm count. The sperms may, however, be damaged by highspeed centrifugation. Whereas the chances with homologous insemination are high in sterility due to mechanical causes, the success in oligospermia is uncertain. Some authors disclaim any success. Statistics of the success range between 0 and 100% and are not comparable because of the different material (ZANARTU, 1960; SCHELLEN, 1957). In Zurich the procedure has been abandoned. In any case the physician will do well to undertake this tiresome procedure, which is a great strain to the married couple, only if the couple expressly requests it.

In *hypogonadism*, it is only possible to achieve fertility by hormone therapy in the case of hypogonadotropic hypogonadism.

Secondary hypogonadotropic hypogonadism can at present be treated with human gonadotropin until normal incretory *and* excretory functions of the testes are restored. Only a few well-examined cases have so far been described.

In a hypophysectomized man with partial pituitary insufficiency and receiving cortisone and androgen substitution, in whom azoospermia developed 2 years after the operation and spermatogenesis was arrested at the spermatid stage, treatment with a weekly dose of 5 mg of human FSH derived from pituitaries for 13 weeks resulted in restoration of normospermia and a sperm count of 61 million/ml (GEMZELL, 1964). A diabetic with a total hypophysectomy whose sperm count fell from 480 million to 0 within 3 months, and whose testis biopsy showed only Sertoli cells and a few remaining spermatogonia responded well to HMG. After 65 daily injections of 5 mg HMG, active spermatogenesis with primary spermatocytes and spermatozoa was demonstrable (MCLEOD, 1966). A probably less purified preparation (Pergonal) obtained from human menopausal urine was used at that time. Recently, clear-cut morphological differences between the immature Sertoli cell of hypogonadotropic hypogonadism and the adult Sertoli cell after treatment with HMG was demonstrated by electron microscopy (DE KRETSER, 1972). Whereas in gonadotropin deficiency inter-Sertoli cell junctions are absent, they appear in the adult Sertoli cells, suggesting that the Sertoli cells have a crucial role in the development of spermatogenesis.

HMG alone has no success on undeveloped testes in congenital secondary hypogonadism (idiopathic eunuchism). On the other hand, preliminary treatment for 6 months with HCG (2000 U three times weekly) resulted in normal development to slight hypertrophy of the Leydig cells and in spermatogenesis to the spermatid stage. Administration of HMG at this point, in addition to HCG (12.5 U of HMG i.m. three times weekly with continuation of HCG in the previous dosage), achieved at least focally complete spermatogenesis with mature spermatozoa in the testis biopsy after 8 weeks (LYTTON, 1966). Complete maturation of the spermatozoa takes about 70 days. Androgens alone or normally functioning Leydig cells can maintain the early stages of spermatogenesis, whereas FSH is necessary for the terminal phases of maturation.

Normal spermatozoa were demonstrated in the ejaculate of 12 of 38 patients with azoospermia and low or absent gonadotropins; in these 12, the testis biopsy showed only Sertoli cells, spermatogonia and some spermatocytes before treatment. Pregnancy occurred in 4 cases. No success was achieved in those where there were no spermatogonia in the testis biopsy. A dose of 75 IU HMG* i.m. every other day is recommended; only if the semen volume is less than 1.5 ml does the treatment, which lasts 80–120 days, have to be combined with HCG, 2500 IU i.m. every other day (LUNENFELD, 1967). Neither the optimal dosage nor the chances of success are known yet. Large amounts of FSH are necessary to initiate spermatogenesis, but maintenance can be achieved by much lower FSH levels, which HCG might also possess (MCLEOD, 1972).

On the other hand, no success can be achieved with HMG and HCG in cases of oligospermia where there is no evidence of reduced gonadotropins (MROUEH, 1967; MCLEOD, 1967).

The very expensive combined replacement therapy with HMG and HCG for hypopitui-

* One ampoule Pergonal or Humegon containing 75 IU FSH and 75 IU LH.

tarism or for hypogonadotropic hypogonadism is only justified when fertility can be achieved, at least temporarily, in the patient. Development of the technique for storing human semen in the deep freeze will increase the chances for hypophysectomized patients to produce children.

In other forms of hypogonadism, hormone therapy can correct signs of failure and metabolic disorders, but not sterility. The use of HCG, and probably also of HMG, is not justified in infertility with oligospermia or normospermia. Gonadotropin from animal serum (PMS, pregnant mare serum) leads to the formation of antibodies within 2 months, and suppresses FSH and LH effects. The testosterone rebound, meaning an attempt to influence oligospermia favorably by producing azoospermia with temporary high doses of testosterone (150 mg weekly) is useless or even harmful. The first critical reports recently published about therapeutic trials with human FSH (HMG) show negative results. Treatment with clomiphene, which increases the gonadotropins, has not been successful in the male either.

Two of the numerous other therapeutic propositions are mentioned here: desiccated thyroid, in a daily dose of 0.2 g, has been considered a means of overcoming sterility for many years. Re-examination, however, has shown that it is only of use in hypothyroidism. Vitamins B_1, E, and A in particular have been recommended. Apart from the fact that avitaminosis occurs only rarely in the human in Western countries, careful studies have shown no effect of vitamin A on oligospermia.

This is not the place for discussing the ethics and psychological problems of heterologous insemination, i.e. insemination with semen of a donor known only to the physician and not to the married couple. The legal principles are manifold and still not fully explained. The legal problems in the European countries and in the USA have been reviewed by SCHELLEN (1957). This procedure is more widely accepted in the United States and in the Scandinavian countries, and is performed by recognized clinics.

Heterologous artificial insemination has not been approved by the Churches. In Switzerland, general opinion is reserved. All the problems have recently been reviewed by GLATTHAAR (1971).

Although the treatment of sterility is so seldom succesful, thorough investigation and consideration of the therapeutic possibilities are to be encouraged. If the physician merely adopts the vague attitude that not much can be done for the patient this produces worrying uncertainty in the patients and drives them into the hands of medical and non-medical quacks. In addition, the childless married couple can be greatly helped by the declaration that investigational possibilities are exhausted and a diagnosis of untreatable sterility has to be made, as they can readjust to the possibility of adopting a child.

4. Induced Reversible Sterility

Induced reversible sterility in the man as a means of birth control is still in the experimental stages. Auto-immunization of the man with testicular tissue inhibits spermatogenesis for just a few weeks (MANCINI, 1965). Substances with an alkylating and cytostatic action do not work specifically enough on spermatogenesis. Hexamethyl phosphoramide has been used in insects and results in long-lasting sterility. Derivatives of hydrazine act very slowly (JACKSON, 1966). The action of a dinitropyrrol on the different stages of maturation have been thoroughly investigated, and the late stages have been found to be the most sensitive. Intermittent administration of the substance in the rat produces reversible sterility (PATANELLI, 1964). Substances acting on different phases of maturation of the sperm are in the first stage of investigation (JACKSON, 1970). Progesterone and other natural steroid hormones can inhibit the capacitation (see p. 482) of sperms and lead to a reversible sterility, an effect which has been observed in animals (SEGAL, 1968). Progesterone influences the uterine mucosa, which prevents the sperms from becoming fertile (see p. 482 and Chap. X, p. 623).

H. Impotence, Satyrism, Perversion

Impotence usually signifies impotentia coeundi, whereas impotentia generandi implies sterility, which is a better term. Impotence is most often due to psychogenic disorders. It is rarely due to vascular diseases, neurological or endocrine factors. Impotence can result from lipiodol arachnoiditis and is an early sign of Buerger's disease. Antihypertensive drugs, especially ganglion-blocking substances such as guanethidine and psychotropic drugs such as phenothiazines may cause impotence.

True endocrine impotence can occur in all forms of hypogonadism with androgen deficiency. However the hypogonadal man is, usually quite unaware of his functional loss since he has no need for it. However, even without manifestations of failure, impotence is a common disorder in advanced age, in hypo-

and hyperfunctional states of the thyroid gland and adrenal cortex, and finally in diabetes mellitus where, as in paraplegics, androgen function is not impaired but neurological disturbances can be detected (FAERMAN, 1972). There have been attempts to differentiate between "constitutional" and "psychogenic" impotence by the urinary excretion of testosterone (COOPER, 1970).

The conflict causing psychogenic impotence can be concealed and may also occur in a person with no neurotic tendencies. It is often astounding what can be achieved with common sense and a brief course of psychotherapy. A treatment with testosterone (25 – 50 mg daily) for 1 – 2 weeks is permissible for the diagnosis ex juvantibus between impotence due to endocrine factors and psychogenic impotence. Stimulation of the libido over a long period can be detrimental if the conflict is not resolved, since the drive is increased but cannot function normally without removal of the inhibition. Testosterone propionate is sometimes useful as an adjuvant. High doses must be used (25–50 mg, 2–3 times weekly, or 250–500 mg depot testosterone i.m. every other week.

Ejaculatio praecox is due to psychological factors and is not an endocrinopathy. Psychotherapy is recommended in the first place. Often the simple means of more frequent sexual intercourse or the application of a cream with a local anesthetic action on the gland can help.

Treatment with 1 mg stilbestrol daily is advocated for excessive sexual drive which is socially unacceptable (satyrism, perversion). The anti-androgen, cyproteron acetate, is also effective in a daily oral dose of 100–200 mg, and it has the advantage of not producing unpleasant side-effects such as gynecomastia and testicular atrophy (see p. 472) (LASCHET, 1968; OTT, 1968).

I. Gynecomastia

Gynecomastia, i.e. increase in male mammary tissue, is a symptom which occurs most frequently in hypogonadism but does also arise in other endocrinopathies and in non-endocrine diseases. HALL (1959) has compiled a thorough and critical monograph on gynecomastia. It occurs frequently in a mild form, and is thought to be histologically demonstrable in 40% of men.

Hormonal regulation of growth of the mammae is discussed in Chap. XI (see p. 694). In addition to estrogens, progesterone, prolactin, growth hormone, and other substances also promote growth of the mamma. These include testosterone, (see p. 475), adrenocortical hormones (desoxycorticosterone, total adrenocortical extracts), and even steroids related to the digitalis compounds. The possibility of conversion of individual steroids and substances within the body must be considered. Chorionic gonadotropin can lead to gynecomastia through stimulation of estrogen production in the testes. Nervous factors also influence growth of the mammae. Drugs which inhibit the hypothalamus (chlorpromazine, reserpine) can result in the release of prolactin and thus cause gynecomastia, probably by inhibiting PIF (see Chap. II, p. 30). Elevated serum prolactin levels have been detected only in patients with gynecomastia due to tranquilizers or reserpine (TURKINGTON, 1972). Finally, sensitivity of the end-organs to hormonal influences must be taken into account, as unilateral gynecomastia or idiopathic gynecomastia could not otherwise be explained. The fact that the factory workers concerned with the production of estrogens and are exposed to them do not all develop gynecomastia, and not all to the same degree, indicates that sensitivity of the end-organ has a decisive effect on the pathogenesis of gynecomastia. An increase in estrogen excretion is found in only one third of patients with gynecomastia. The initiating factor in pubertal gynecomastia seems to be associated with the growth hormone. The patho-anatomical changes in gynecomastia are due to proliferation of the efferent ducts and a simultaneous increase in the surrounding connective tissue. The efferent ducts are not only lengthened and branched, but are also increased in number. True glandular lobules, on the other hand, develop only in rare cases, such as in the presence of endocrine active teratomas (see p. 490f.). The epithelium of the excretory ducts often consists of many layers, and papillary-like protrusions may develop. Proliferation of the basket cells may also occur. Treatment with estrogens leads to pronounced epithelial proliferation. Histologically, the epithelia occasionally show signs of secretion. On the other hand, milk or colostrum which can be expressed are only exceptionally produced. Two types of gynecomastia can be differentiated according to the connective tissue surrounding the excretory ducts. In one form, the excretory ducts are enclosed by coats of loose connective tissue which is surrounded by a tissue rich in collagen fibers. In the second form, the excretory ducts are embedded directly in a connective tissue with a very high content of collagenous fibers with no loose tissue. These two types are probably different stages of development, the type with loose sheaths of connective tissue depicting the active phase, and the type with collagen-rich connective tissue only being the inactive phase. Small inflammatory infiltrations

consisting of lymphocytes, plasma cells, histiocytes and occasionally leukocytes as well can almost always be demonstrated.

Table 8. Occurrence of gynecomastia. (Modified after BRONSTEIN, 1950; and HALL, 1959)

A. Physiological gynecomastia (see Chap. XIX)
1. Gynecomastia in the newborn
2. Pubertal gynecomastia, transitory and persistent forms
B. Gynecomastia in endocrinopathies
1. Hypogonadism (castration, Klinefelter's syndrome, liver diseases, malnutrition, involution)
2. Testicular tumors (p. 490)
3. Feminizing adrenal tumors (p. 387)
4. Hyperthyroidism
5. Tumors of the anterior pituitary: acromegaly, chromophobe adenoma, craniopharyngeoma
6. Hermaphroditism
C. Drug-induced gynecomastia
1. Gonadotropins
2. Estrogens
3. Testosterone (p. 475)
4. Desoxycorticosterone
5. Digitalis
6. Isoniazid, alpha-methyl dopa, amphetamine, reserpine, chlorpromazine, certain anti-androgens.
D. Gynecomastia in non-endocrine diseases
1. Leprosy
2. Leukemia
3. Diseases of the nervous system (injuries to the spinal cord and intercostal nerves, syringomyelia, Friedreich's ataxia)
4. Bronchial carcinoma
5. Osteoarthropathy
6. Uremia
7. Chronic hemodialysis
E. Familial
F. Idiopathic

The differential diagnosis includes pseudogynecomastia or lipomastia, which arise from hypertrophy of fatty tissues. It also includes inflammatory processes and true tumors which may arise spontaneously or after hormone therapy. Palpation is essential for the diagnosis since pseudo-gynecomastia cannot always be differentiated from true gynecomastia by mere observation. Palpation will always reveal firm glandular tissue in true gynecomastia, and glandular enlargement can vary from the size of a pea to that of a fist. After the history has been obtained, with special reference to any drugs taken, examination must then be directed to the testes and pituitary. The testes must be carefully palpated. Even in the absence of a palpable testicular tumor, it is advisable to perform a simple pregnancy test on the urine to exclude chorial carcinoma. In every case of gynecomastia, a lateral X-ray of the sella must be taken, liver and thyroid must be investigated, and thorax screening should be performed to exclude a bronchial carcinoma.

1. Pubertal Gynecomastia

Refer to Chap. XIX, p. 1041.

2. Gynecomastia in Endocrinopathies

Gynecomastia in hypogonadism occurring after starvation and involution and in the presence of testicular tumors has been discussed on p. 490. Gynecomastia occurring with rare feminizing adrenal cortical tumors is dealt with on p. 378. Gynecomastia is occasionally seen in hyperthyroidism, sometimes arising only with the remission after ^{131}I therapy. There may be some connection between gynecomastia and renourishment after starvation.

3. Gynecomastia Produced by Drugs

Gynecomastia can arise temporarily during treatment of cryptorchidism and hypogonadotropic eunuchoidism with chorionic gonadotropin. Estrogens used therapeutically in men (carcinoma of the prostate, satyrism) almost always result in gynecomastia. It can be prevented by prophylactic X-ray irradiation (HAURI, 1971). Testosterone propionate, and more frequently methyl testosterone, may produce gynecomastia during treatment of primary hypogonadism. Conversion of testosterone into estrogens within the body has been considered the cause. Development of gynecomastia after administration of desoxycorticosterone and total adrenocortical extracts has not been explained. Digitalis glycosides, spironolactones, isoniazid, alpha-methyl dopa, amphetamine, reserpine, and chlorpromazine (see p. 558) may occasionally cause gynecomastia.

4. Gynecomastia in Nonendocrine Diseases

Gynecomastia is thought to occur commonly and in severe forms in leprosy. Leprosy does, however, cause hypogonadism at the same time, so that this may also be involved in the etiology of the gynecomastia. Estrogen excretion does not appear to be increased. Gynecomastia has been described in paraplegics after traumatic damage to the spinal cord. It has also been observed after injury to the intercostal nerves during thoracoplastic operations and in bronchial carcinoma. Half of all patients with osteoarthropathy have gynecomastia. The clubbed fingers and the gynecomastia may regress after vagotomy. There have been isolated reports of gynecomastia occurring in other diseases, such

as ulcerative colitis, erythrodermia, and lymphogranuloma. Coincidental occurrence cannot be excluded for the time being. A curious observation is the frequent occurrence of gynecomastia in chronic hemodialysis (one third to one half of all cases). It is explained as a form induced by renourishment, such as was seen in the Second World War, due to the restoration of gonadotropin secretion. Increased FSH and LH secretion is assumed (FREEMAN, 1968; SCHMITT, 1968; SAWIN, 1973).

5. Familial Gynecomastia

Familial occurrence has been reported. It is probably inherited through a recessive or autosomal dominant gene with sex-linkage and slight hypogonadotropic hypogonadism (ROSEWATER, 1965) (see p. 470).

6. Therapy

Any known cause must be removed. Surgical removal is indicated when the gynecomastia is conspicuous or painful. This, however, should not be done until 1 or 2 years after puberty, i.e. not before the 18–19th year. Spontaneous regression is still possible during this time. Local application of testosterone can help against pain. Regression of the gynecomastia is not to be expected with testosterone therapy. X-rays before estrogen therapy can prevent gynecomastia. Intensive radiotherapy may be tried therapeutically (HAURI, 1971).

K. Syndromes of Testicular Hormonal Overproduction

The overproduction of testicular hormones occurs only in tumors with the exception of one well studied case (GOLDFINE, 1971). Besides this, compensatory hyperplasia of one testis is seen in semicastration or underdevelopment of the other testis (LARON, 1969). Total testosterone secretion remains unchanged. For increased testosterone secretion relative to age see pubertas praecox, Chap. XIX, p. 1047.

1. Neoplasms of the Testis

Neoplasms of the testis account for about 0.5 to 1% of malignant tumors in the male. They can arise from the testis itself or from its coats and appendages. True tumors of the testis are not uncommonly endocrine active. On the other hand, tumors of the testicular coats and appendages, in particular the so-called adenomatoid tumors of the epididymis, paratesticular fibromas, fibrosarcomas, liposarcomas and rhabdomyosarcomas, produce no endocrine symptoms (SCHRÖDER, 1970). We are therefore limiting our discussion to true testicular neoplasms.

In the classification of these tumors, there are certain differences between the American (DIXON, 1952) and European nomenclature (V. ALBERTINI, 1955; COLLINS, 1964) most commonly in use. We prefer the classification recommended by COLLINS as it offers the advantage of being especially simple, while permitting a clear classification of teratomas (Table 9). The whole group of malignant teratomas corresponds to so-called chorial carcinomas (V. ALBERTINI), whereas the anaplastic malignant teratoma corresponds to the embryonal carcinoma of DIXON.

Table 9. Classification of tumors of the testis, epididymis and neighboring structures. (After COLLINS and PUGH, 1964)

1. *Tumors of the testis*
Seminomas
Teratomas:
Teratomas, differentiated forms (TD)
Malignant teratomas, intermediary form (MTI)
A: with differentiated or organoid areas (MTI A)
B: without differentiated or organoid areas (MTI B)
Malignant teratomas, anaplastic forms (MTA)
Malignant teratomas, trophoblastic forms (MTT)
Combination tumors:
seminoma and teratoma in the same testis
Sertoli-cell tumors
Interstitial-cell tumors
Orchioblastomas
Malignant lymphomas
Metastases
Various types and unclassifiable tumors
2. *Tumors of the epididymis, testicular coats and spermatic cord*
Adenomatoid tumor
Tumor of connective tissue and muscle;
Benign: fibroma, leiomyoma, lipoma, etc.
Malignant: embryonal sarcoma, rhabdomyosarcoma, fibrosarcoma, leiomyosarcoma, etc.
Metastases
Various types and unclassifiable tumors

The relative frequency of the individual tumors of the testis is indicated in Table 10, which has been derived from 995 tumors of the testis (COLLINS, 1964). Seminomas, teratomas and combination tumors consisting of seminomas and teratomas, which are almost without exception malignant, together constitute 86% of tumors of the testis. The other group, however, also includes predominantly malignant tumors, such as orchioblastomas, malignant lymphomas and metastases, and these together

make up a further 8% of the total number of testicular tumors. Thus, benign tumors of the testis are exceptionally uncommon.

a) Seminomas

Seminomas are the most common tumors of the testis. They occur most often in men between the 30th and 50th year. They are more common on the right side. Furthermore, they occur more frequently in imperfectly descended testes than in normally descended testes. On rare occasions, both testes are involved. Seminomas combined with a teratoma are not uncommon (see combination tumors). Histologically, they consist of clusters of cells, loosely arranged. These clusters vary in size and are separated by narrow septa in which groups of small lymphocyte-like round cells are embedded. The tumor cells themselves are fairly large, possessing ill-defined clear cytoplasmic edges and rather large, roundish nuclei. The tumor cells thus appear similar to germ cells, from which they also arise. According to the degree of differentiation of the tumor cells banal seminomas, which are more frequent, are separated from the less common spermatocytic forms. Giant cells are not infrequently found in seminomas. Occasionally a tuberculoid reaction is seen in the interstitial and surrounding tissues. Seminomas metastasize relatively late. They are very radiosensitive and have a good prognosis. As a rule, metastases first arise in the iliac and paraaortal abdominal lymph nodes. They may also occur in the inguinal lymph nodes.

Seminomas may cause a slight rise in urinary gonadotropin levels. This rise, however, is not so pronounced as with teratomas. SYMINGTON and WALLACE (1964) found an average rise from 20 units HPG/day in the healthy subject to 36 units HPG/day in patients with seminomas. These values can temporarily increase even further after surgery. The rise is attributed to a loss of normal testicular tissue, and thus to inadequate inhibition of the pituitary.

b) Teratomas

True teratomas are somewhat less common than seminomas. They occur in younger age groups than seminomas, and are particularly common in men in the third decade. Teratomas are also more frequently found on the right side. Bilateral teratomas are extremely rare.

Teratomas of the testis are almost without exception malignant, in contrast to the corresponding ovarian tumors. Thus, mature testicular tumors which may appear to be benign must be examined carefully for undifferentiated, malignant areas. COLLINS and PUGH (1964) found only 13 benign lesions among 322 true teratomas (4%).

Table 10. Frequency of the different types of tumors among a total of 995 testicular tumors. (After COLLINS and PUGH, 1964)

	Number of cases	%
Seminomas	400	40
Teratomas	322	32
Combination tumors (seminoma + teratoma)	136	14
Sertoli-cell tumors	6	2
Interstitial-cell tumors	14	
Orchioblastomas	8	8
Malignant lymphomas	66	
Metastases	6	
Various types and unclassifiable tumors	37	4
Total	995	100

The frequency of different malignant forms can be seen in Table 10. Since quite undifferentiated areas can be demonstrated in practically all malignant testicular tumors, COLLINS and PUGH (1964) classify teratomas according to their most highly differentiated areas, even when these are only localized. In the intermediate form (MTI), more or less differentiated tissue can be demonstrated. Fully mature tissue or organoid structures can be found in intermediate form A (MTI A), and immature tissue only and no organoid structures can be seen in intermediary form B (MTI B). An anaplastic teratoma (MTA) can only be referred to in the presence of undifferentiated, mainly cancerous cell masses, a picture corresponding to so-called embryonal carcinomas. A trophoblastic malignant teratoma (MTT) can only be assumed to be present when villous-like structures can be recognized. Syncytial giant cells alone are not sufficient to justify this diagnosis since isolated giant cells can be found in almost all forms of malignant teratomas and even in seminomas.

Malignant teratomas have a considerably worse prognosis than seminomas. Metastases were confirmed at surgical removal of testicular tumors in almost one sixth of the patients in the COLLINS and PUGH series (1964). Half of the 322 patients with teratomas died within the next 4 years, 62% of them in the first postoperative year.

In contrast to seminomas, teratomas are often endocrine-active. Like placental tissue, they produce mainly chorionic gonadotropin (HCG). They also produce estrogens and progesterone. Large amounts of chorionic gonadotropin may be produced and may cause a positive result in immunological pregnancy reactions

even in a dilution of 1:10 (Gravindex) or for 20–40000 IU/l (Pregnosticon). HCG, progesterone and estrogens together cause gynecomastia associated with character changes in the patient, with awakening of maternal instincts. Pregnandiol, a derivative of progesterone, is excreted. Estrogen excretion is also elevated. Demonstration of a massive urinary excretion of chorionic gonadotropin confirms the diagnosis of a malignant teratoma. It is also of importance in the follow-up of the patient.

Table 11. Frequency of the different types of teratomas among 322 teratomas. (After COLLINS and PUGH, 1964)

	Cases	%
Differentiated teratomas (TD)	13	4
Malignant teratomas		
Intermediate form A (MTI A)	181	56
Intermediate form B (MTI B)	101	31.5
Anaplastic form (MTA)	16	5
Trophoblastic form (MTT)	11	3.5
Total	322	100

c) Combination Tumors (Teratomas and Seminomas)

Tumors consisting of teratomas and seminomas are relatively common, as is shown in Table 2. Combinations are possible with all forms of teratomas, and their frequency corresponds roughly to that of the teratomas without seminomas. At present there is no definite explanation for this tumor combination. v. ALBERTINI (1943) speaks of reactive seminomas in teratomas, but this reaction cannot be explained merely by abnormal hormonal stimulation. The prognosis of such combined tumors is somewhat more favorable than that of teratomas without seminomas. The endocrine activity corresponds to the type of the teratoma.

d) Sertoli-Cell Tumors (Androblastomas)

Sertoli-cell tumors are some of the least well-defined testicular tumors. They are rare in the human, and large investigation series are therefore not available. The tumors vary widely in the histological picture. Typical Sertoli-cell tumors are relatively small and are sharply defined. Microscopy shows strands of cells and gland-like formations, their epithelia being reminiscent of Sertoli cells. Such tumors occur in both normally situated and undescended testes. They only rarely metastasize.

In addition to these tumors with more or less typical Sertoli formations, other tumors also arise with proliferation of the interstitial tissue. For this reason, DIXON and MOORE (1952) subdivided these tumors, which were collectively described as androblastomas by TEILUM (1946), into 3 forms, namely diffuse stromal forms, mixed stromal and glandular forms, and true tubular forms. MORRIS and SCULLY (1958) refer to the corresponding ovarian tumors simply as Sertoli-Leydig cell tumors since the combination of a proliferation of glandular tubes and stromal constituents is very typical. Such tumors are a rarity in man, whereas they are the most common form of testicular tumor in dog.

Sertoli-cell tumors may be endocrine-active, forming estrogens or androgens. Gynecomastia may occur. COLLINS and SYMINGTON (1964) found elevated gonadotropin urinary excretion in one case. They themselves, however, also raised the question of whether this tumor might be a teratoma and not a Sertoli-cell tumor.

Besides the Sertoli-cell tumors tumor-like proliferations of hypoplastic tubules occur. Single hypoplastic tubules may be found in normally developed and descended testes, at least in young adults (HEDINGER, 1968). In undescended testes, however, such tubules may form whole fields, the so-called hypoplastic zones. In the syndrome of testicular feminization transitions to the so-called *tubular adenoma* PICK are found.

Furthermore, tumors of dysgenetic gonads, the so-called *gonadoblastomas*, are a special group (SCULLY, 1953; SIEBENMANN, 1961). The same applies to similar tumors, which can be seen in the gonadal crest and in testicular tissue on the opposite side in the mixed testicular dysgenesis where there is a chromosome mosaic (SALLE, 1969) (see also Chap. XII, p. 722).

e) Interstitial Cell Tumors

These tumors also occur only rarely. In large statistical series they account for about 1–2% of testicular tumors. A total of rather more than 100 cases has so far been reported in the medical literature. It is probable that some of these cases are not true tumors but rather hyperplasias of Leydig cells or of Leydig-like cells of adrenocortical tissue in the congenital adrenogenital syndrome. This is especially true of the few observations of so-called "bilateral Leydig-cell tumors" and of the "unilateral tumors" in children with pseudopubertas praecox, in whom the clinical symptoms do not regress after orchidectomy but may even worsen (see p. 366).

α) True Leydig-Cell Tumors

Tumors of the Leydig cells are usually small, attaining the size of a walnut or a hen's egg at

the most. They are encapsulated. Their cut surface is yellowish-brown. The tumors are composed of cells very similar to Leydig cells. They may enclose brown pigment and crystals in addition to fatty substances. Cells and nuclei are very polymorphous, which suggests malignancy. The tumors are, however, generally benign. Only very few cases of confirmed malignant Leydig-cell tumors with formation of metastases have been observed. The histological picture, however, hardly differs from that of the benign tumor.

Leydig-cells can produce testosterone and estradiol (see p. 451). Leydig-cell tumors can result in an enormous increase in 17-ketosteroid excretion. Amounts of 1 g/day have been measured. Biologically active steroids (androgens), on the other hand, are increased to a lesser degree. Nevertheless, 470 µg/day testosterone glucuronide and 345 µg/day testosterone sulfate have been measured. The latter substance is assumed to originate directly from the tumor. Testosterone production is estimated to be 50 mg/day with a plasma testosterone concentration of 1.9 µg/100 ml, and dehydro-epitestosterone sulfate production to be 2.27 g/day (LIPSETT, 1966). Androgen overproduction does not become clinically manifest in the mature man. In contrast, in infancy it results in pseudopubertas praecox with disproportional stunted stature and extremely pronounced male sexual characteristics.

Increased amounts of estrogens may also be excreted in the presence of Leydig-cell tumors. Increased production becomes clinically manifest with the development of gynecomastia. Natural and synthetic estrogens can cause Leydig-cell tumors in certain breeds of mice. The mechanism of this process is not clear.

β) Adrenocortical-Like Interstitial Cell Tumors

These tumors are histologically quite similar to adrenocortical tissue, and a few authors have therefore stated that they are due to proliferation of ectopic components of the adrenal cortex frequently demonstrated in the testis of the newborn. The picture may often vary in the same tumor. Such tumors may lead to pseudopubertas praecox in the child and to gynecomastia in the adult. Formation of estrogens has been chemically demonstrated. In infantile cases with pseudopubertas praecox, the possibility of a congenital adrenogenital syndrome with hyperplasia of ectopic adrenocortical tissue must always be considered v. ALBERTINI concluded that these adrenocortical-like tumors are large-cell hypernephroid varieties of true Leydig-cell tumors, since this type of hypernephroid picture can often also be observed in tumors of other endocrine organs. THEILUM considers all Leydig cell-like tumors, as well as Sertoli-cell adenomas or so-called tubular adenomas as various differentiation forms of tumors of a single common parent cell, the androblast. He calls all these tumors androblastomas.

As long as large numbers of individual observations continue to be unavailable and our knowledge of the histogenesis and endocrine activity of these tumors remains inadequate, it is advisable to classify such neoplasms by purely morphological criteria. Thorough hormonal investigations are indicated in all cases before removal of the tumor. It is possible that the nature of these tumors may become more apparent following treatment with hormones. Thus, response to HCG, LH, or ACTH, i.e. regression with estrogens or cortisone due to inhibition of the adenotropic hormone, may perhaps throw some light on the type of tumor.

f) Orchioblastoma (Adenocarcinoma of infancy, yolk sac tumor, endodermal sinus tumor)

Orchioblastomas occur only in infants. The histological picture is very uniform. It shows a partly papillary and partly trabecular structure of a clear-cell adenocarcinoma. Some authors consider orchioblastomas to belong to the teratoma group. The orchioblastoma is an exceptionally typical tumor with no signs of endocrine activity. The tumors are malignant, but the prognosis appears to be somewhat more favorable than that of the corresponding teratoma (KARLY, 1968; THEILUM, 1971).

g) Malignant Lymphomas

Malignant lymphomas, lymphosarcomas and reticulosarcomas can occur in the testis as in most organs. However, primary forms are uncommon. The possibility of metastases or primary generalized forms must always be recognized.

h) Metastases

The frequency given in Table 2 refers entirely to surgical material. In postmortems, metastases in the testis are not so uncommon and are found especially in cases of leukemia and malignant lymphoma. However, other tumors, e.g. small-cell bronchus carcinoma, also metastasize to the testis occasionally. Endocrine symptoms can hardly be expected since the basic malignant disease is usually predominant.

L. Methods of Investigation

1. Clinical Signs

The history and clinical examination alone can show whether hypogonadism is present or not because no other hormone influences features to such an extent as the male sexual hormones. Fertility in all likelyhood excludes hypogonadism.

Attention must be paid to the development of the male sexual characteristics, to the type of voice, pubic and axillary hair, skeletal maturation and body stature. If the size of the testes is normal, i.e. >15 cm^3, then hypogonadism is highly improbable, even if the external masculine appearance is not impressive. It must be remembered that the testes first increase in size with the onset of puberty and are infantile prior to this (see Chap. XIX, p.1035).

2. Examination of the Semen

In contrast to incretory testicular function, spermatogenesis can only be assessed by examining the semen or testicular biopsy if the ability to reproduce does not already indicate a normal state. In the presence of small testes, especially if they are greatly reduced in size, spermatogenesis is always severely impaired or completely absent. Milder degrees of infertility, however, can only be recognized through examination of the semen.

The simplest form of semen examination can be performed by the general practitioner, whereas more detailed morphological and physiochemical assessment requires special laboratories. Examination of the semen is essential to the investigation of hypogonadism and every case of sterility. For psychological reasons it is usually not possible before early adulthood.

Procedure. Abstinence from sexual intercourse is required for 3–5 days prior to the test (see p. 483). Semen can be obtained either by masturbation or by coitus interruptus, and should be collected in a clean dry glass vessel. Condoms are not suitable for collecting the semen since the substances contained in the rubber and powder can damage the spermatozoa. If possible semen should be examined 30–60 minutes after ejaculation. The time of collection must be indicated in every case as the time since ejaculation is important in the assessment of motility. Assessment of fertility is also possible from material sent by post to special laboratories.

Macroscopic Examination. Volume in ml, appearance and viscosity (measured by immersing a platinum loop), must be noted. When the viscosity is normal, semen drops from the loop, whereas pathological semen sticks to the loop or forms threads.

Microscopic Investigation. Motility: after thorough shaking, a few drops of the semen are put on a glass slide and covered with a cover slip. The motility is assessed under high magnification, focusing on the uppermost layer. The motile and immotile spermatozoa should be counted in a quarter of the total field so that the motility can be evaluated as a precentage. The degree of motility can also be indicated by 1–4 crosses. Special laboratories employ so-called revival methods with special solutions for assessing motility. This distinguishes between apparently dead spermatozoa and true necrospermia.

Sperm Count. After thorough mixing, semen is aspirated into a leukocyte pipette up to the 0.5 mark. A diluting solution is then aspirated to the 11 mark. HOTCHKISS employs a 4% sodium bicarbonate solution with 1% phenol as a diluting solution. According to JOEL, it is an advantage to use physiological saline with 1% ZIEHL'S carbol fuchsin, which stains the spermatozoa and allows them to be easily detected. After 2 min of mixing, a counting chamber for counting erythrocytes is filled. Five large squares with 16 small squares, each i.e. 80 small squares in all, are counted. The number of spermatozoa counted in the 5 large squares is multiplied by a million. This gives the sperm count per cubic centimeter. The mean of 2–3 counts must be taken.

Morphology. Assessment of the percentage of pathological forms of spermatozoa requires special knowledge. Whereas there is no difficulty in differentiating between normal forms and obviously pathological forms such as double formations, giant head types, and amorphous forms, it is difficult to recognize certain intermediate forms (Fig. 21). According to HOTCHKISS, a smear must be prepared for morphological assessment. It is then fixed in 95% methyl alcohol for 5 min and stained with hematoxylin-eosin or special stains.

Other Investigations. Numerous other investigations such as measurement of pH, revival tests, resistence tests, estimation of fructose, citrate, and different enzymatic activities are performed in specialized laboratories.

The clinical significance of these tests is not yet certain. It is, however, still maintained that the fertility of a sperm is not conclusively proven, even if all the methods of investigation show it to be normal.

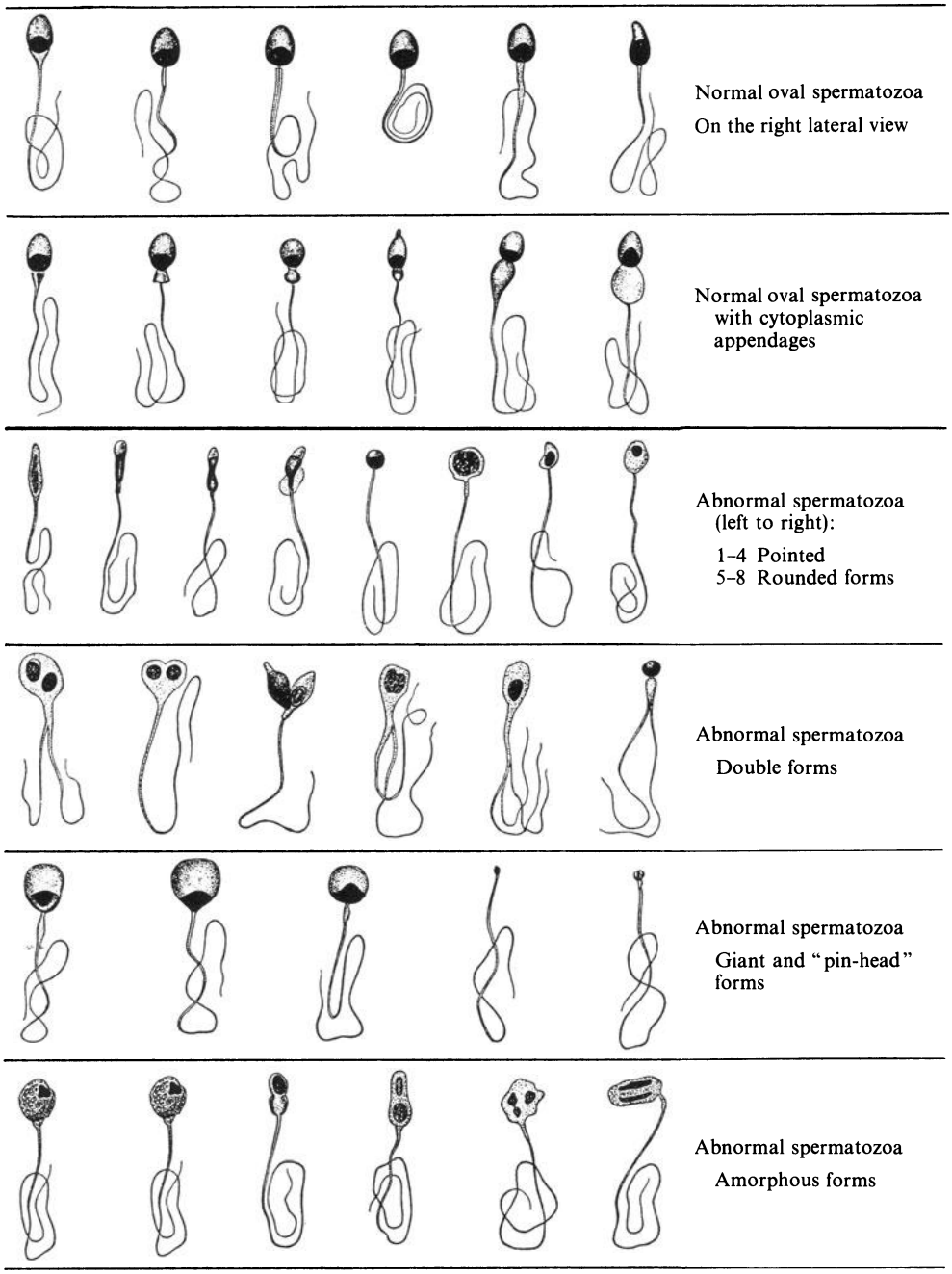


Fig. 21. Morphology of spermatozoa. (After R. S. HOTCHKISS, Infertility in Men, Springfield: Ch. C. Thomas 1952)

Evaluation. The volume of semen, sperm count, motility and morphology of spermatozoa are important in assessment of the fertility of the patient.

The volume normally lies between 2.5 and 3.5 cm^3. Deviations to 0 and as high as 10 or 15 cm^3 are possible. Too large a volume is unfavorable for fertility since the concentration of spermatozoa falls. The normal sperm count is considered to be 40–120 million per cm^3. Fertility is reduced with counts below 40 million. Fertility is highly improbable with values under 20 million. It cannot be emphasized strongly enough that a single assessment of the sperm count is no indication of the degree of fertility. The sperm count can vary by several. 100 % within a week even in healthy men. Repeated assessments must be made and at least 3 are required before fertility can be assessed.

Normal fertility necessitates at least 40% motile spermatozoa, counted 30–60 min after ejaculation.

Furthermore, at least 60% of the spermatozoa should be morphologically normal, and in all, not more than 2% of other cells, such as leukocytes, epithelial cells or cells of spermatogenesis, should be present (see also p. 455).

3. Testis Biopsy

Indication. Testis biopsy is absolutely essential for the exact diagnosis of male hypogonadism (see p. 460). Azoospermia in the presence of testes of normal size is an absolute indication for a biopsy since it is necessary for the detection of obstructions of the seminiferous ducts which can be corrected by surgical means. The biopsy indicates the prognosis in cases of oligospermia and saves costly and useless attempts of treatment in therapeutically hopeless cases (HEDINGER, 1971). In contrast to semen investigation, it can also be performed during childhood.

Procedure. Biopsy of the testis is only a minor surgical procedure and can be performed on outpatients. Strict asepsis is of course essential. We prefer to hand over our patients to the surgeon in spite of the technical simplicity of this minor procedure. The physician should not take the responsibility for postoperative hemorrhages, which do occasionally arise. Admission to hospital for 2 days is advisable for anxious patients.

We usually do unilateral biopsies. American authors recommend bilateral biopsies as a precaution, although divergences are seldom found.

Operative Technique. The skin between the umbilicus and middle of the thigh is shaven and disinfected. The scrotum is held so that the skin is stretched above the testis to be examined. The testis should not be rotated and the incision should be made along the ventral side. Local anesthesia with nerve block of the funiculus at the level of the site of exit from the external inguinal ring ensures complete freedom from pain. With local anesthesia alone, slight pain may be felt on incision of the albuginea, which cannot be infiltrated because of the risk of damaging the testicular tissue lying below.

After nerve block and local anesthesia of the skin and subcutaneous tissue, a longitudinal incision of approximatively 1.5 cm is made in the skin at the base of the scrotum. Such an incision causes much less discomfort than the more distal transverse incision. The scrotal skin can then be spread with a selfholding eye retractor. The tunica vaginalis is also anesthesized. After a cut of 3 to 5 mm through the tunica albuginea, a small amount of testicular tissue can be made to emerge by slight pressure on the testis, and a $4 \times 3 \times 2$ mm piece of tissue can be easily excised. Care must be taken that this piece of tissue is not compressed, torn or shaken. Therefore we prefer to excise with a scalpel rather than with scissors. The biopsy is immediately placed into the fixing solution, because evaluation is impossible if it becomes dried out. We suture the tunica albuginea with the finest cotton thread to avoid any oozing of blood, while the tunica vaginalis is not sutured to permit any drainage to occur. Some authors suggest the albuginea not to be sutured to avoid subsequent pain.

The scrotal skin is closed with one or two catgut sutures. A collodium bandage is applied and a suspensory is worn over the bandage for a week. On the day of operation one should stay quietly at home and on the following day there should be very little discomfort.

Handling of the Testicular Tissue. For histological examination tissues of 2 mm in diameter are sufficient. Amerişan authors recommend Zenker's fluid with a 5% addition of glacial acetic acid, Helly's fluid, or Bouin's fluid (ROULET, 1948) for fixation. We had good results with Stieve's mixture.

Stieve's mixture:	
Saturated aqueous sublimate solution	76 ml
Formaldehyde (40 %)	20 ml
Acetic acid, glacial	4 ml

After 24 hours at the latest, the biopsies have to be put into 70% alcohol where they can remain for a longer period of time without damage. The fixation in Stieve's solution occurs quickly and thoroughly and prevents the washout of cells, especially from the seminiferous tubules. This frequently happens when biopsies are fixed in the usual 4% aqueous formalin solution.

Interpretation. Histological examination enables reliable assessment of the state of development, of any degenerative process, of germinal epithelium, and of the Leydig cells. On the other hand, however, it is not always possible to differentiate between primary testicular damage and secondary changes on the basis of the histology alone. Biopsy must therefore be complemented by appropriate hormone estimations. Although the biopsy provides information from only a very small section of the whole testis, results obtained are very informative, as has been shown in various testicular changes (CLAVADETSCHER, 1970; HEDINGER, 1971).

4. Testosterone Estimations and Functional Evaluation of the Hypothalamus-Pituitary-Gonadal-Axis

M. ZACHMANN

Since only about 1% of the produced testosterone is excreted as unmetabolised testosterone glucuronide, the quantities of testosterone in urine are low even in mature males. Nevertheless urinary testosterone determinations are useful in special cases or for research studies because very specific gaschromatographic techniques (CURTIUS, 1970) are available. Normal values in adult males range from 30 to 160 µg/24 h with a mean value of 72 µg/24 h (age 18–38 years), with other methods normal values have even a range between 15–520 µg/24 h (see p. 379). Above the age of 40 years, urinary testosterone tends to decrease gradually. In prepubertal boys, urinary testosterone remains undetectable (below 0,5 µg/24 h) up to a bone age of about 8 years. Thereafter, it gradually increases and reaches adult levels at a bone age of about 16 years. In adult females, urinary testosterone is about 12 µg/24 h or less.

For clinical purposes, the gaschromatographic techniques for testosterone determination in urine are too time consuming and the less specific estimations of plasma testosterone are more practical. Among others, various competitive protein binding techniques (FISCH, 1973) and radioimmunoassay methods (FOREST, 1973) are useful for the estimation of testosterone in plasma. Normal plasma testosterone values in adult males are 612 ± 172 ng/100 ml (range 300–1000 ng/100 ml). In women, the levels are much lower (35 ± 22 ng/100 ml, range 10–60 ng/100 ml). The same is true in prepubertal children where extremely low values (6.6 ng/100 ml, boys and girls) are found up to a bone age of about 8 years. Only in newborns and infants up to about 3 months there is a sex difference, the values in boys being considerably higher than in girls (FOREST, 1973).

The Leydig-cell function may be evaluated by determining testosterone in urine or plasma after stimulation with human chorionic gonadotropin. In this respect, various test procedures have been proposed and most frequently, HCG has been given repeatedly and plasma testosterone determined before and after several days or weeks of HCG administration. When 800–5000 units of HCG were given for 5 days in prepubertal boys, plasma testosterone increased from 26 to 431 ng/100 ml (RIVAROLA, 1970). In adult males, the post HCG values are accordingly higher and may reach levels up to 2000 ng/100 ml.

Especially during prepuberty, a more specific evaluation according to the biological age of the subject studied may be obtained by giving only a single dose of HCG (5000 units/m^2 of bodysurface area) and determining urinary or plasma testosterone 2, 4 and 6 days after injection (ZACHMANN, 1972). With this procedure urinary testosterone increases logarithmicly with bone age from birth to maturity.

Recently luteinizing hormone releasing hormone (LHRH), a decapeptide elaborated by the hypothalamus, has been synthesized and has become available for testing the gonadotropin secreting capacity of the anterior pituitary (BENER, 1972; KASTIN, 1972; REBAR, 1973; ROTH, 1972; TAMM, 1973).

The combined analysis of HCG, LHRH and possibly also clomiphene (see p. 599) test results presently allows a differentiated evalutation of the hypothalamus-pituitary-gonadal axis and makes a localization of the disorder possible.

For other hormone determinations refer to Chaps. VII, X and XI.

References

Reviews

ROSENBERG, E., PAULSEN, C.A. (eds.): The human testis New York and London: Plenum 1970.

The testis, ed. by JOHNSON, A.D., GOMES, W.R., VANDEMARK, N.L. Vol. I, II, III. New York-London: Academic Press 1970–1971.

VOSS, H.E., OERTEL, G.W. (eds.): Androgene I. Handbook of experimental pharmacology, New Series, Vol. XXXV 3 xv/1. Berlin-Heidelberg-New York: Springer 1973.

Embryology, Anatomy and Histology

ÅNBERG, Å.: The ultrastructure of the human spermatozoon. Acta obstet. gynec. scand. **36**, Suppl. 2 (1957).

BURGOS, M.H., FAWCETT, D.W.: Studies on the fine structure of the mammalian testis. I. Differentation of the spermatids in the cat (Felis domestica). J. biophys. biochem. Cytol. **1**, 287 (1955).

BURNS, R.K.: Urogenital system in analysis of development, hrsg. von B.H. WILLIER, P.A. WEISS und V. HAMBURGER. Philadelphia und London: W.B. SAUNDERS & Co. 1955.

CHRISTENSEN, A.K.: The fine structure of testicular interstitial cells in guinea pigs. J. Cell Biol. **26**, 911 (1965).

— FAWCETT, D.W.: The normal fine structure of opossum testicular interstitial cells. J. biophys. biochem. Cytol. **9**, 653 (1961).

FAWCETT, D.W.: Spermtail structure in relation to the mechanism of movement. In: Spermatozoan mobility (D.W. BISHOP, ed.), Washington, D.C., A.A.A.S. Publ. No 72, p. 147, 1962.

HELLER, C.G., CLERMONT, J.: Kinetics of the germinal epithelium in man. Recent Progr. Hormone Res. **20**, 545—571 (1964).

HORSTMANN, E.: Elektronenmikroskopische Untersuchungen zur Spermiohistogenese beim Menschen. Z. Zellforsch. **54**, 68—89 (1961).

JOST, A.: Influence de la décapitation sur le développement du tractus génital et des surrénales de l'embryon du lapin. C.R. Soc. Biol. (Paris) **142**, 172 (1948).
— Sur le contrôle hormonal de la différenciation sexuelle du lapin. Arch. Anat. micr. Morph. exp. **39**, 577 (1950).
— CHODKIEWICZ, M., MAULÉON, P.: Intersexuality of the calf fetus produced by androgens. Comparison between the fetal hormone responsible for free-martinism and the adult testicular hormone. C.R. Acad. Sci. (Paris) **256**, 274 (1963).
KRETSER, D.M. DE: The fine structure of the testicular interstitial cells in men of normal androgenic status. Z. Zellforsch. **80**, 594 (1967).
LILLIE, F.R.: The free-martin: A study of the action of sex hormones in the fetal life of cattle. J. exp. Zool. **23**, 371 (1917).
MOORE, C.R.: On the role of sex hormones in sex differenciation in the oppossum (Didelphys virginiana). Physiol. Zool. **14**, 1 (1941).
MURAKAMI, M.: Elektronenmikroskopische Untersuchungen am interstitiellen Gewebe des Rattenhodens, unter besonderer Berücksichtigung der Leydigschen Zwischenzellen. Z. Zellforsch. **72**, 139 (1966).
PONSE, K.: La différenciation du sexe et l'intersexualité chez les vertèbres. Lausanne: F. Rouges 1949.
RAYNAUD, A., FRILLEY, M.: Destruction des glandes génitales de l'embryon de souris par une irradiation au moyen des rayons X, à l'âge de 13 jours. Ann. Endocr. (Paris) **8**, 400—419 (1947).
TÖNDURY, G.: Entwicklungsstörungen durch chemische Faktoren und Viren. Naturwissenschaften **42**, 312 (1955).
— Embryologie im Dienste der Krankheitsforschung. Ergebnisse der medizinischen Grundlagenforschung. Stuttgart: Georg Thieme 1956.
TORREY, T.: Intraocular grafts of embryonic gonads of the rat. J. exp. Zool. **115**, 37—58 (1950).
WITSCHI, E.: Migration of the germ cells of human embryos from the yolk sack to the primitive gonadal folds. Contr. Embryol. Carneg. Instn **32**, 67 (1945).
— Génétique et physiologie de la différenciation du sexe. Arch. Anat. micr. Morph. exp. **39**, 215 (1950).
— Embryogenesis of the adrenals and the reproductive glands. Recent Progr. Hormone Res. **6**, 1 (1951).
WOLFF, ET.: Le rôle de hormones embryonaires dans la différenciation sexuelle des oiseaux. Arch. Anat. micr. Morph. exp. **39**, 426 (1950).

Biochemistry

ANDERSON, R.M., LIAO, S.: Selective retention of dihydrotestosterone by prostatic nuclei. Nature **219**, 277 (1968).
BAIRD, D.T., HORTON, R., LONGCOPE, C., TAIT, J.F.: Steroid prehormones. Perspect. Biol. Med. **11**, 384 (1968).
— — — — Steroid dynamics under steady-state conditions. Recent Progr. Hormone Res. **25**, 611 (1969).
BAULIEU, E.E., CORPÉCHOT, C., DRAY, F., EMILIOZZI, R., LEBEAU, M.C., MAUVAIS-JARVIS, P., ROBEL, P.: An adrenal-secreted androgen: dehydroisoandrosterone sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates. Recent Progr. Hormone Res. **21**, 411 (1965).
BODEL, P.T., DILLARD, M., BONDY, P.K.: The mechanism of steroid-induced fever. Ann. intern. Med. **69**, 875 (1968).
BONDY, P.K., COHN, G.L., GREGORY, P.G.: Etiocholanolone fever. Medicine (Baltimore) **44**, 249 (1965).
BRUCHOVSKY, N., WILSON, J.D.: The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostrate *in vivo* and *in vitro*. J. biol. Chem. **243**, 2012 (1968).
CAMACHO, A.M., MIGEON, C.J.: Studies on the origin of testosterone in the urine of normal adult subjects and patients with various endocrine disorders. J. clin. Invest. **43**, 1083 (1964).
COPPAGE, W.S., COONER, A.E.: Testosterone in human plasma. New Engl. J. Med. **273**, 902 (1965).
DAUGHADAY, W.F., MARIZ, I.K.: The binding of steroid hormones by plasma proteins. In: Biological activities of steroids in relation to cancer, G. PINCUS, E.P. VOLLMER, eds, p. 61. New York: Academic Press 1960.
DICZFALUSY, E., LAURITZEN, CHR.: Oestrogene beim Menschen. Berlin-Göttingen-Heidelberg: Springer 1961.
DORFMAN, R.I., FORCHIELLI, E., GUT, M.: Androgen biosynthesis and related studies. Recent Progr. Hormone Res. **19**, 251 (1963).
— SHIPLEY, R.A.: Androgens. New York: Wiley & Sons 1956.
DRIESSEN, O., VOÛTE, P.A., VERMEULEN, A.: A description of two brothers with permanently raised non esterified aetiocholanolone blood level. Acta endocr. (Kbh.) **57**, 177 (1968).
EIK-NES, K.B. (ed.): The androgens of the testis. New York: Dekker 1970.
— Production and secretion of testicular steroids. Recent Progr. Hormones Res. **27**, 517 (1971).
FISHMAN, L.M., SARFATY, G.A., WILSON, H., LIPSETT, M.B.: The role of the testis in oestrogen production. Ciba Coll. Endocr. **16**, 156 (1957).
FRENCH, F.S., BAGGETT, B., WYCK, J.J. VAN, TALBERT, L.M., HUBBARD, N.R., JOHNSTON, F.R., WEAVER, R.P., FORCHIELLI, E., RAO, G.S., SARDA, I.R.: Testicular feminization: clinical and morphological studies. J. clin. Endocr. **25**, 661 (1965).
GANDY, H.M., MOODY, C.B., PETERSON, R.E.: Androgen levels in ovarian and adrenal venous plasma. Proc. VI th. Panamer. Congr. Endocr. 1965. Excerpta medica Found. (Amsterdam) internat. Congr. Series **112**, 223 (1966).
GEORGE, J.M., WOLFF, S.M., DILLER, E., BARTTER, F.C.: Recurrent fever of unknown etiology: Failure to demonstrate association between fever and plasma unconjugated etiocholanolone. J. clin. Invest. **48**, 558 (1969).
HALL, P.F.: Endocrinology of the testis. In: The testis, JOHNSON, A.D., GOMES, W.R., VANDEMARK, N.L., eds. London-New York: Academic Press 1970.
HOLLANDER, N., HOLLANDER, V.P.: The microdetermination of testosterone in human spermatic vein blood. J. clin. Endocr. **18**, 966 (1958).
HORTON, R., TAIT, J.F.: *In vivo* studies of steroid dynamics-androstenedione and testosterone. In: Androgens in normal and pathological conditions. Proc. 2nd Symp. Steroid Hormones, A. VERMEULEN and D. EXLEY, eds., p. 199. Amsterdam: Excerpta medica Found. 1966.
HUDSON, B., COGHLAN, J.P., DULMANIS, A.: Testicular function in man. Ciba Coll. Endocr. **16**, 140 (1967).
JOHNSON, A.D., GOMES, W.R., VANDEMARK, N.L.: The testis, vol. II, Biochemistry. London-New York: Academic Press 1970.
KAPPAS, A., HELLMAN, L., FUKUSTRIMA, D., GALLAGHER, T.F.: The thermogenic effect and metabolic fate of etiocholanolone in man. J. clin. Endocr. **18**, 1043 (1958).
— PALMER, R.H., GLICKMAN, P.B.: Steroid fever. Amer. J. Med. **31**, 167 (1961).
KLEIN, E.: Das Testosteron. Die Struma. 13. Symp. der Dtsch. ges. f. Endokrinologie. Berlin-Heidelberg-New York: Springer 1968.
LIPSETT, M.B., KORENMAN, S.G.: Androgen metabolism. J. Amer. med. Ass. **190**, 757 (1964).
— WILSON, H., KIRSCHNER, M.A., KORENMAN, S.G., FISHMAN, L.M., SARFATY, G.A., BARDIN, C.W.: Studies on Leydig cell physiology and pathology: secretion and metabolism of testosterone. Recent Progr. Hormone Res. **22**, 245 (1966).
MCKERNS, K.W. (ed.): The gonads. Amsterdam: North Holland 1969.

MEEKER, C.I.: Plasma testosterone levels in pregnancy. Excerpta medica Found. (Amsterdam) internat. Congr. Series **111**, 174 (1966).
MERCIER, C.: Specificity of a plasma testosterone-binding globulin. Excerpta medica Found. (Amsterdam) internat. Congr. Series **111**, 269 (1966).
MOSEBACH, K.O.: Verteilung, Bindung und Metabolite von Testosteron im Säugetierorganismus. 13. Symp. Endokrinologie. Berlin-Heidelberg-New York: Springer 1968.
NEHER, R.: Biosynthese des Testosterons. In: Das Testosteron. Die Struma. 13. Symp. der Dtsch. Ges. f. Endokrinologie. Berlin-Heidelberg-New York: Springer 1968.
PRUNTY, F.T.G.: Androgen metabolism in man—some current concepts. Brit. med. J. **1966 II**, 605.
RIVAROLA, M.A., FOREST, M.G., MIGEON, C.J.: Testosterone, androstenedione and dehydroepiandrosterone in plasma during pregnancy and at delivery. Concentration and protein binding. J. clin. Endocr. **28**, 34 (1968).
ROSENFIELD, R.L.: Plasma unbound androgens in hirsutism. 52. Meet. Endocr. Soc. (1970), Abstr. 75.
SANDBERG, A.A., SLAUNWHITE, W.R. JR.: Metabolism of 4-C^{14}-testosterone in human subjects. I. Distribution in bile, blood, feces and urine. J. clin. Invest. **35**, 1331 (1956).
SCHMIDT, H.: Testosteronausscheidung bei männlichen Personen unter normalen und pathologischen Bedingungen. Acta endocr. (Kbh.), Suppl. 128 to Vol. **58** (1968).
SLAUNWHITE, W.R. JR., SANDBERG, A.A.: Metabolism of 4-C^{14}-testosterone in human subjects. II. Isolation of metabolites from plasma. J. biol. Chem. (1957).
TAMM, J.: Testosterone. Proc. Work-shop Conf. Tremsbüttel. Stuttgart: Thieme 1967.
— Der Testosteronstoffwechsel beim Menschen. Biosynthese des Testosterons. Dtsch. med. Wschr. **92**, 1983 (1967).
— Der Testosteronstoffwechsel beim Menschen. Testosteron im Plasma und Testosteron-Produktionsraten. Dtsch. med. Wschr. **92**, 2037 (1967).
— Der Testosteronstoffwechsel beim Menschen. Der periphere Stoffwechsel des Testosterons. Dtsch. med. Wschr. **92**, 2080 (1967).
VERMEULEN, A.: Testosterone in plasma. Advanc. Biosciences **2**, 103 (1969).
VIDA, J.A.: Androgens and anabolic agents. Chemistry and pharmacology. New York-London: Academic Press 1969.
WEST, C.D., REICH, H., SAMUELS, L.T.: Urinary metabolites after intravenous injection of human subjects with testosterone. J. biol. Chem. **192**, 219 (1951).
WILLIAMS-ASHMAN, H.G.: Biochemical features of androgen action. Advanc. Biosciences **2**, 200 (1969).
WILSON, J.D., WALKER, J.D.: The conversion of testosterone to 5α-androstan-17β-ol-3-one (dihydrotestosterone) by skin slices of man. J. clin. Invest. **48**, 371 (1969).

Physiology

DORFMAN, R.I., SHIPLEY, R.A.: Androgens. Biochemistry, Physiology and clinical significance. New York: Wiley 1956; London: Chapman&Hall 1956.
EIK-NES, K.B., HALL, P.F.: Secretion of steroid hormones *in vivo*. Vitam. and Horm. **23**, 153 (1965).
HUSEBY, R.A., DOMINGUEZ, O.V., SAMUELS, L.T.: Function of normal and abnormal testicular interstitial cells in the mouse. Recent Progr. Hormone Res. **17**, 1 (1961).
LACY, D., PETTITT, A.J.: Sites of hormone production in the mammalian testis, and their significance in the control of male fertility. Brit. med. Bull. **26**, 87 (1970).
LEACH, R.B., MADDOCK, M.O., TOKUYAMA, I., PAULSEN, C.A., NELSON, W.O.: Clinical studies of testicular hormone production. Recent Progr. Hormone Res. **12**, 377 (1956).
LIPSETT, M.B., WILSON, H., KIRSCHNER, M.A., KORENMAN, S.G., FISHMAN, L.M., SARFATY, G.A., BARDIN, C.W.: Studies on Leydig cell physiology and pathology: Secretion and metabolism of testosterone. Recent Progr. Hormone Res. **22**, 245 (1966).
MADDOCK, W.O., NELSON, W.O.: The effect of chorionic gonadotropin in adult men: increased estrogen and 17-ketosteroid excretion, gynaecomastia, Leydig-cell stimulation and seminiferous tubule damage. J. clin. Endocr. **12**, 985 (1952).
MANN, T.: Male sex hormone and its role in reproduction. Recent Progr. Hormone Res. **12**, 353 (1956).
PINCUS, G.: The physiology of ovarian and testis hormones. In The hormones, vol. III. New York: Acad. Press 1955.
TAMM, J.: Testosterone. Proc. Workshop-Conference, Tremsbüttel 1967. Stuttgart: Thieme 1968.

Pituitary Control of Testosterone Production

BELL, E.T., LORAINE, J.A.: Recent research on gonadotrophic hormones. Edinburgh-London: Livingstone 1967.
BURGER, H.G., BAKER, H.W.G., HUDSON, B., TAFT, H.P.: Gonadotropic and gonadal function in the normal adult male. In: Gonadotropins, SAXENA, BELING and GRANDY, eds. New York: Wiley-Interscience 1972.
BUTT, W.R.: The chemistry of the gonadotrophins. Springfield, USA: Thomas 1967.
DAVIS, T.E., LIPSETT, M.B., KORENMANN, S.G.: Suppression of testosterone production by physiologic doses of 2α-methyldihydrotestosterone propionate. J. clin. Endocr. **25**, 476 (1965).
EIK-NES, K.B.: Effects of gonadotrophins on secretion of steroids by the testis and ovary. Physiol. Rev. **44**, 609 (1964).
— Factors influencing the secretion of testosterone in the anaesthetized dog. Ciba Coll. Endocr. **16**, 120 (1967).
EVANS, J.I., MACLEAN, A.M., ISMAIL, A.A.A., LOVE, D.: Circulating levels of plasma testosterone during sleep. Proc. roy. Soc. Med. **64**, 841 (1971).
FISHMAN, L.M., SARFATY, G.A., WILSON, H., LIPSETT, M.B.: The role of the testis in oestrogen production. Ciba Coll. Endocr. **16**, 166 (1967).
FRANCHIMONT, P.: Radioimmuno assay of gonadotropines. Advanc. Biosciences **1**, 19 (1969).
— Application of radioimmuno assay of gonadotrophins in clinical research. In: IRVINE, W.I., Reproductive endocrinology. Edinburgh and London: Livingstone 1970.
HALL, P.F., EIK-NES, K.B.: The action of gonadotropic hormones upon rabbit testis *in vitro*. Biochim. biophys. Acta (Amst.) **63**, 411 (1962).
HELLER, C.G., NELSON, W.O.: The testes-pituitary relationship in men. Recent Progr. Hormone Res. **3**, 329 (1948).
HUDSON, B., COGHLAN, J.P., DULMANIS, A.: Testicular function in man. Ciba Coll. Endocr. **16**, 140 (1967).
LEONARD, J.M., LEACH, R.B., COUTURE, M., PAULSEN, C.H.: Plasma and urinary follicle-stimulating hormone levels in oligospermia. J. clin. Endocr. **34**, 209 (1972).
MCCULLAGH, E.P., HRUBY, F.J.: Testis-pituitary interrelationsship: relative inability of testosterone to reduce urinary gonadotropin in eunuchoid men. J. clin. Endocr. **9**, 113 (1949).
NELSON, W.O.: Interrelations of gonatrophic and gonadal hormones in the regulation of testicular functions. Ciba Coll. Endocr. **4**, 271 (1952).
SAUNDERS, F.J.: Effects of steroids on pituitary gonadotropin and fertility. Recent Progr. Hormone Res. **20**, 395 (1964).
SAXENA, P.B., BELING, C.G., GRANDY, H.M. (eds.): Gonadotropins. New York: Wiley-Interscience 1972.
SLAUNWHITE, W.R. JR., SANDBERG, A.A., JACKSON, J.E., STAUBITZ, W.J.: Effects of estrogen and HCG on androgen synthesis of human testis. J. clin. Endocr. **22**, 992 (1962).

THIEL, D. H. VAN, SHERINS, R. J., MYERS, G. H., JR., VITA, V. T. DE, JR.: Evidence for a specific seminiferous tubular factor affecting follicle-stimulating hormone secretion in man. J. clin. Invest. **51**, 1009 (1972).
VAN DE WIELE, R. L., MCDONALD, P. C., GURPIDE, E., LIEBERMAN, S.: Studies on the secretion and intraconversion of the androgens. Recent Progr. Hormone Res. **19**, 275 (1963).
WOLSTENHOLME, G. E. W., KNIGHT, J. (eds.): Gonatropins: physico-chemical and immunological properties. Ciba Found. Study group 22. London: Churchill 1965.

Effects and Mode of Action of Testosterone

ARVILL, A.: Effects of testosterone on the metabolism of the isolated levator ani muscle of the rat. Acta endocr. (Kbh.), Supp. **122**, 1 (1967).
BAULIEU, E. E., LASNITZKI, I., ROBEL, P.: Metabolism of testosterone and action of metabolites on prostate glands grown in organ culture. Nature (Lond.) **219**, 1155 (1968).
BUTENANDT, A., GÜNTHER, H., TURBA, F.: Zur primären Stoffwechselwirkung des Testosterons. Hoppe Seylers Z. physiol. Chem. **322**, 28 (1960).
DORFMAN, R. I.: Mechanism of action of steroid hormones: Androgens. In: C. A. VILLEE and L. L. ENGEL, Mechanism of action of steroid hormones. Oxford: Pergamon Press 1961.
EDER, H. A.: The effects of sex hormones on serum lipids and lipoproteins. In: G. PINCUS: Hormones and atherosclerosis. New York: Academic Press 1959.
FANG, S., ANDERSON, K. M., LIAO, S.: Receptor proteins for androgens. On the role of specific proteins in selective retention of 17β-hydroxy-5α-androstan-3-one by rat ventral prostate *in vivo* and *in vitro*. J. biol. Chem. **244**, 6584 (1969).
FREY-WETTSTEIN, M., CRADDOCK, C. G.: Wirkung von Testosteron auf lymphatisches und hämatopoietisches System der Ratte. Schweiz. med. Wschr. **100**, 1984 (1970).
KASSENAAR, A. A. H., QUERIDO, A., HAAK, A.: Effects of anabolic steroids on nucleic acid and protein metabolism. In: F. GROSS, Protein metabolism. Berlin-Göttingen-Heidelberg: Springer 1962.
KENNEDY, B. J.: Stimulation of erythropoiesis by androgenic hormones. Ann. intern. Med. **57**, 917 (1962).
KOCHAKIAN, CH. D.: The protein anabolic effect of steroid hormones. Vitam. and Horm. **4**, 255 (1946).
— Androgen regulation of nucleic acid and protein biosynthesis in the prostate. In: Biology of the prostate and related tissues. Nat. Cancer Inst. Monogr. **63**, 411 (1963).
MANN, T.: The biochemistry of semen and of the male reproductive tract, 2nd ed. London: Methuen 1964.
MEIER, C. J., KRÄHENBÜHL, CH., DESAULES, P. A.: Etude comparative des effects sexuels des androgènes naturels. Acta endocr. (Kbh.) **43**, 27 (1963).
QUERIDO, A., KASSENAAR, A. A. H.: Nitrogen-retaining steroids and their application in disease. Advanc. metabol. Disorders **2**, 79 (1965).
ROCHEFORT, H., BAULIEU, E. E.: Mécanisme d'action des androgènes dans la prostate et la vésicule séminale. In: Le testicule et l'hypogonadisme masculin. Paris: L'Expansion 1967.
RUNDLES, R. W.: Action of anabolic steroids on red-cell production. In: F. GROSS, Protein metabolism. Berlin-Göttingen-Heidelberg: Springer 1962.
RUSS, E. M., EDER, H. A., BARR, D. P.: Influence of gonadal hormones on protein-lipid relationship in human plasma. Amer. J. Med. **19**, 4 (1955).
SALMON, W. D., BOWER, P. H., THOMPSON, E. Y.: Effect of protein anabolic steroids on sulfate incorporation by cartilage of male rats. J. Lab. clin. Med. **61**, 120 (1963).
SIITERI, P. K., WILSON, J. D.: Dihydrotestosterone (DHT) and benign prostatic hypertrophy (BPH) in man. 52. Meet. Endocr. Soc. (1970), Abstr. 78.
STRAUSS, J. S., POCHI, P. E.: The human sebaceous gland: Its regulation by steroidal hormones and its use as an end organ for assaying androgenicity *in vivo*. Recent Progr. Hormone Res. **19**, 385 (1963).
TAMM, J., VOIGT, K. D.: Einige klinisch relevante Ergebnisse der neueren Androgenforschung. Schweiz. med. Wschr. **101**, 1078 (1971).
TVETER, K. J., ATTRAMANDAL, A.: Selective uptake of radioactivity in rat ventral prostate following administration of testosterone-1,2-^{3}H. Methodological considerations. Acta endocr. (Kbh.) **59**, 218 (1968).
VIDA, J. A.: Androgens and anabolic agents. Chemistry and pharmacology. New York-London: Academic Press 1969.
VILLEE, C., FUJII, T.: Effects of testosterone on RNA metabolism im prostate and seminal vesicle. In: TAMM, Testosterone. Stuttgart: Thieme 1968.
WILKINS, L., FLEISCHMANN, W.: The influence of various androgenic steroids on nitrogen balance and growth. J. clin. Endocr. **6**, 383 (1946).
WILLIAMS-ASHMAN, H. G.: Incorporation of amino acids into protein by cell-free extracts of the prostate gland: effect of testicular hormones and polyribonucleotides. Nat. Cancer Inst. Monogr. **12**, 281 (1963).
WILSON, J. D.: Regulation of protein synthesis by androgens and estrogens. In: F. GROSS, Protein metabolism. Berlin-Göttingen-Heidelberg: Springer 1962.
YIELDING, K. L., TOMKINS, G. M.: The regulation of enzyme structure and function by steroid hormones (Editorial). Studies on the interaction of steroid hormones with glutamic dehydrogenase. Amer. J. med. **33**, 1 (1962).

Formation of Semen

BISHOP, D. W.: Sperm motility. Phys. Rev. **42**, 1 (1962).
CLERMONT, Y.: Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. Physiol. Rev. **52**, 198 (1972).
GEMZELL, C., KJESSLER, B.: Treatment of infertility after partial hypophysectomy with human pituitary gonadotrophins. Lancet **1964I**, 644.
HALL, P. F.: Endocrinology of the testis. In: The testis, JOHNSON, A. D., GOMES, W. R., VANDEMARK, N. L., eds. London-New York: Academic Press 1970.
HOHLWEG, W.: Die Wirkung von Testosteron auf die Spermiogenese im Sinne eines Gewebshormons. In: Gewebs- und Neurohormone. Physiologie des Melanophorenhormons. 8. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1962.
JOHNSEN, S. G.: The mechanisms involved in testicular degeneration in man. Acta endocr. (Kbh.), Suppl. **124**, 17 (1967).
LANMAN, J. T.: Delays during reproduction and their effects on the embryo and fetus. I. Aging of sperm. New Engl. J. Med. **278**, 993 (1968).
MACLEOD, J., PAZIANOS, A., RAY, B.: The restoration of human spermatogenesis and of the reproductive tract with urinary gonadotropins following hypophysectomy. Fertil. Steril. **17**, 7 (1966).
MANN, T.: Biochemistry of semen and of the male reproductive tract. London: Methuen 1964.
NEUMANN, F., ELGER, W., STEINBECK, H., BERWORDT-WALLRABE, R. VON: Antiandrogene. In: Testosteron-Struma. 13. Symp. Dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.
STEINBERGER, A., STEINBERGER, E.: Factors affecting spermatogenesis in organ cultures of mammalian testes. J. Reprod. Fertil., Suppl. **2**, 117 (1967).
STEINBERGER, E.: Hormonal control of mammalian spermatogenesis. Physiol. Rev. **51**, 1 (1971).

Male Hypogonadism

Reviews

ALBERT, A., UNDERDAHL, L.O., GREENE, L.F., LORENZ, N.: Male hypogonadism. Proc. Mayo Clin. **28**, 409, 557, 698 (1953); **29**, 131, 317, 368 (1954); **30**, 31 (1955).

CLAYTON, B.E., HUTCHINSON, J.S.M., HYDE, R.D., LONDON, D.R., MILLS, I.H., PRUNTY, T.T.G.: A study of the classification of male hypogonadism with observations on the control of 17-oxosteroid precursors from the testes and adrenals. J. Endocr. **34**, 587 (1960).

DECOURT, J., GILBERT-DREYFUS: Le testicule et l'hypogonadisme masculin. Actualités endocr. Paris: L'Expansion 1967.

HELLER, C.G., NELSON, W.O.: Classification of male hypogonadism and a discussion of the pathologic physiology, diagnosis and treatment. J. clin. Endocr. **8**, 345 (1948).

HOWARD, R.P., SNIFFEN, R.C., SIMMONS, F.A., ALBRIGHT, F.: Testicular deficiency: a clinical and pathological study. J. clin. Endocr. **10**, 121 (1950).

NOWAKOWSKI, H.: Der Hypogonadismus im Knaben- und Mannesalter. Ergebn. inn. Med. Kinderheilk. **12**, 219 (1959).

OBERNDORFER, A.: Die inneren männlichen Geschlechtsorgane. In: F. HENKE and O. LUBARSCH, Handbuch der speziellen pathologischen Anatomie. Vol. 6, part 3, p. 427. Berlin: Springer 1931.

PRADER, A.: Hypogonadismus beim Knaben. Schweiz. med. Wschr. **1955**, 737.

RIMOIN, D.L., BORGAONKAR, D.S., ASPER, S.P., JR., BLIZZARD, R.M.: Chromatin-negative hypogonadism in phenotypic men. Amer. J. Med. **44**, 225 (1968).

TONUTTI, E., WELLER, O., SCHUCHARDT, E., HEINKE, E.: Die männliche Keimdrüse. Struktur-Funktion-Klinik. Grundzüge der Andrologie. Stuttgart: Thieme 1960.

VOIGT, K.D., NOWAKOWSKI, H.: Steroid excretion in hypogonadism. Acta endocr. (Kbh.), Suppl. **31**, 181 (1957).

The Different Forms of the Male Hypogonadism

Symptomatology of Androgen Failure, Castration, Anorchia

Primary Testicular Insufficiency

ABEYARATNE, M.R., AHERNE, W.A., SCOTT, J.E.S.: The vanishing testis. Lancet **1969 II**, 822.

BECKER, K..L., ALBERT, A.: Urinary excretion of follicle-stimulating and luteinizing hormones. J. clin. Endocr. **25**, 962 (1965).

BLEULER, M.: Endokrinologische Psychiatrie. Stuttgart: Georg Thieme 1955.

— Endokrinologische Psychiatrie. In: Psychiatrie der Gegenwart, Bd. I/IB. Berlin-Göttingen-Heidelberg: Springer 1964.

BOWER, B.F.: Pituitary enlargement secondary to untreated primary hypogonadism. Ann. intern. Med. **69**, 107 (1968).

CAWADIAS, A.P.: Male eunuchism, considered in the light of the historical method. Proc. roy. Soc. Med. **39**, 501 (1946).

FRANCHIMONT, P.: La sécretion des gonadotrophines chez le sujet normal et dans différentes conditions artificielles et pathologiques. Schweiz. med. Wschr. **101** (1971).

GADERMANN, E.: Regressive Wirbelsäulenveränderungen bei doppelseitiger Hodenatrophie und Anorchie. Verh. dtsch. Ges. inn. Med. **58**, 400 (1952).

GLENN, J.F., MCPHERSON, H.T.: Anorchism: Definition of a clinical entity. J. Urol. (Baltimore) **105**, 265 (1971).

HAMILTON, J.B.: The role of testicular secretions as indicated by the effects of castration in man and by studies of pathological conditions and the short life span associated with maleness. Recent Progr. Hormone Res. **3**, 257 (1948).

— Effect of castration in adolescent and young adult males upon further changes in the proportions of bare and hairy scalp. J. clin. Endocr. **20**, 1309 (1960).

— BUNCH, L.D.: Serum inorganic phosphorus levels in eunuchism and in feeble-minded states. J. clin. Endocr. **18**, 180 (1958).

— — MESTLER, G.E., IMAGAWA, R.: Effect of orchiectomy upon chemical constituents of blood in young mature males, with special reference to serum P. J. clin. Endocr. **16**, 301 (1956).

KELCH, R.P., JENNER, M.R., WEINSTEIN, R., KAPLAN, S.L., GRUMBACH, M.M.: Estradiol and testosterone secretion by human, simian, and canine testes, in males with hypogonadism and in male pseudohermaphrodites with the feminizing testes syndrome. J. clin. Invest. **51**, 824 (1972).

KELLER, P.J.: The renal clearance of follicle-stimulating and luteinizing hormone in postmenopausal women. Acta endocr. (Kbh.) **53**, 225 (1966).

— Hormonal studies in endocrine disease. Germ. med. Mth. **13**, 389 (1968).

KIRSCHNER, M.A., JACOBS, J.B., FRALEY, E.E.: Bilateral anorchia with persistent testosterone production. New Engl. J. Med. **282**, 240 (1970).

KOCH, W.: Über die russisch-rumänische Kastraktensekte der Skopzen. Veröff. Kriegs- u. Konstit. path. **2**, H. 7 (1921).

LABHART, A., COURVOISIER, B.: Osteoporose bei Eunuchismus. Helv. med. Acta **17**, 475 (1950).

LANGE, J.: Die Folgen der Entmannung Erwachsener. Leipzig: Thieme 1934.

LINDBERG, B.J.: Psycho-infantilism. Nord. Med. **49**, 838 (1953).

LYON, R.P.: Torsion of the testicle in childhood. J. Amer. med. Ass. **178**, 702 (1961).

NOWAKOWSKI, H.: Hypogonadismus im Knaben- und Mannesalter. Ergebn. inn. Med. Kinderheilk. **12**, 219 (1959).

PELIKAN, E.: Gerichtlich-medizinische Untersuchungen über das Skopzentum in Rußland. Translated by Iwanoff. Giessen: Ricker 1876.

PITTARD, E.: Les Skoptzy. Paris: Masson & Cie. 1934.

TAMM, J., SCHMIDT, H., STARCEVIC, Z., KLOSTERHALFEN, H.: Urinary excretion of testosterone, 17-Ketosteroids and oestrogens in male patients following orchiectomy. In: Androgens. Excerpta medica Found. (Amsterdam) 1966.

WAGENSEIL, F.: Chinesische Eunuchen. Z. Morph. Anthrop. **32**, 415 (1953).

WOLF, CH.: Die Kastration bei sexuellen Perversionen und Sittlichkeitsverbrechen des Mannes. Basel: Schwabe 1934.

Primary Hypogonadism in Hereditary Diseases and in Degenerative Syndromes

CLARKE, B.G., SHAPIRO, S., MONROE, R.G.: Myotonia atrophicans with testicular atrophy: urinary excretion of interstitial cell-stimulating (luteinizing) hormone, androgens and 17-ketosteroids. J. clin. Endocr. **16**, 1235 (1956).

DRUCKER, W., BLANC, W.A., ROWLAND, L.P., GRUMBACH, M.M., CHRISTY, N.P.: The testis in myotonic muscular dystrophy: a clinical and pathologic study with a comparison with the Klinefelter syndrome. J. clin. Endocr. **23**, 59 (1963).

DUNN, H.G.: The Prader-Labhart-Willi syndrome: review of the literature and report of 9 cases. Acta paediat. scand., Suppl. **186**, 1 (1968).

JACOBSON, W.E., SCHULTZ, A.L., ANDERSON, J.: Endocrine studies on 8 patients with dystrophic myotonia. J. clin. Endocr. **15**, 801 (1955).

KAPLAN, E., SHWACHMAN, H., PERLMUTTER, A.D., RULE, A., CHAW, K.T., HOLSCAW, D.S.: Reproductive failure in males with cystic fibrosis. New Engl. J. Med. **279**, 65 (1968).
KUHN, E.: Endokrinologische Untersuchungen bei myotonischer Dystrophie. I. Steroidhormonbildendes Endokrinium. Endokrinologie **41**, 153 (1961).
LIPSETT, M.B., WILSON, H., KIRSCHNER, M.A., KORENMAN, S.G., FISHMAN, L.M., SARFATY, G.A., BARDIN, C.W.: Studies on Leydig-cell physiology and pathology: secretion and metabolism of testosterone. Recent Progr. Hormone Res. **22**, 245 (1966).
NOWAKOWSKI, H., LENZ, W.: Genetic aspects in male hypogonadism. Recent Progr. Hormone Res. **17**, 53 (1961).
PRADER, A., LABHART, A., WILLI, H.: Ein Syndrom von Adipositas, Kleinwuchs, Kryptorchismus und Oligophrenie nach myatonieartigem Zustand im Neugeborenenalter. Schweiz. med. Wschr. **86**, 1250 (1956).
REINFRANK, R.F., NICHOLS, F.L.: Hypogonadotrophic hypogonadism in the Laurence-Moon syndrome. J. clin. Endocr. **24**, 48 (1964).
SOHVAL, A.R., SOFFER, L.J.: Congenital familiar testicular deficiency. Amer. J. Med. **14**, 328 (1953).
— — Congenital testicular deficiency. II. Defective Sertoli-cell differentiation in so called „obscure origin". J. clin. Endocr. **13**, 408 (1953).
STEINER, H.: Das Prader-Labhart-Willi-Syndrom. Eine morphologische Analyse. Virchows Arch. Abt. A Path. Anat. **345**, 205 (1968).
THANNHAUSER, S.J.: Werner's Syndrome (progeria of the adult) and Rothmund's Syndrome: two types of closely related heredofamilial atrophic dermatoses with juvenile cataracts and endocrine features: a critical study with five new cases. Ann. intern. Med. **23**, 559 (1945).
VISCHER, D., LABHART, A., PRADER, A., GINSBERG, J.: Das Prader-Labhart-Willi-Syndrom. Syndrom von Myatonie, Oligophrenie, Adipositas, Hypogenitalismus, Hypogonadismus, Diabetes mellitus. In: PFEIFFER, E.F., Handbuch des Diabetes mellitus, vol. II. München: Lehmann 1971.
WEINSTEIN, L.R., KLIMAN, B., SCULLY, R.E.: Familial syndrome of primary testicular insufficiency with normal virilisation, blindness, deafness and metabolic abnormalities. New Engl. J. Med. **281**, 969 (1969).
ZACHMANN, M., PRADER, A.: Funktionsprüfung der Leydig-Zellen bei Kindern und Jugendlichen. Die Wirkung einer einmaligen Injektion von Choriongonadotropin auf die Testosteronausscheidung. Schweiz. med. Wschr. **100**, 538 (1970).

Tubular Insufficiency (see also "Sterility")

Damage due to Inflammation

BUENZLI, H.: Formen granulomatöser Orchitis. Virchows Arch. Abt. A Path. anat. **345**, 33 (1968).
CRAIGHEAD, J.E., MAHONEY, E.M., CRAVER, D.H., NAFICY, K., FREMONT-SMITH, P.: Orchitis due to coxsackie virus group B, type 5. New Engl. J. Med. **267**, 498 (1962).
CRUICKSHANK, B., STUART-SMITH, D.A.: Orchitis associated with sperm-agglutinating antibodies. Lancet **1959 I**, 708.
KABAL, E.A.: Uses of hyperimmune human gamma globulin. New Engl. J. Med. **269**, 247 (1963).
KOCEN, R.S., CRITCHLEY, E.: Mumps epididymo-orchitis and its treatment with cortisone. Brit. med. J. **1961 II**, 20.
PETERSDORF, R.G., BENNETT, I.L.: Treatment of mumps orchitis with adrenal hormones. Arch. intern. Med. **99**, 22 (1957).
RIGGS, S., SANFORD, J.P.: Viral orchitis. New Engl. J. Med. **266**, 990 (1962).
ROSEMAN, B.D.: Mumps orchitis. Clin. Proc. Child. Hosp. (Wash.) **14**, 239 (1958).

Damage due to Elevated Temperature

BORS, E., ENGLE, E.T., ROSENQUIST, R.C., HOLLIGER, V.H.: Fertility in paraplegic males. J. clin. Endocr. **10**, 381 (1950).
HANLEY, H.G., HARRISON, R.G.: The nature and surgical treatment of varicocele. Brit. J. Surg. **50**, 64 (1962/63).
STEINBERGER, E., DIXON, W.J.: Some observations on the effect of heat on the testicular germinal epithelium. Fertil. Steril. **10**, 578 (1959).
ZEITLIN, A.B., COTTRELL, TH.L., LLOYD, F.A.: Sexology of the paraplegic male. Fertil. Steril. **8**, 337 (1957).

Damage due to Ionizing Rays

COURSEY, E. DE: Pathologic anatomy of lethal ionizing radiation: late developments. Amer. J. Path. **23**, 889 (1947).
HEMPELMANN, L.H., LISCO, H., HOFFMAN, J.G.: The acute radiation syndrome: a study of nine cases and a review of the problem. Ann. intern. Med. **36**, 279 (1952).
LEROY, G.U.: Medical sequelae of atomic bomb explosion. J. Amer. med. Ass. **134**, 1143 (1947).
LIEBOW, A.A., WARREN, S.: Early effects of radiation. Amer. J. Path. **23**, 888 (1947).
MACLEOD, J., HOTCHKISS, R.S., SITTERSON, B.W.: Male infertility after sterilization by nuclear radiation. J. Amer. med. Ass. **187**, 637 (1964).
OAKES, W.R., LUSBAUGH, C.C.: Course of testicular injury following accidental exposure to nuclear radiations. Report of a case. Radiology **59**, 737 (1952).
PLATT, W.R.: Effects of radioactive phosphorus (P 32) on normal tissues. Arch. Path. **43**, 1, (1947).
SCHINZ, H.R., SLOTOPOLSKY, B.: Der Röntgenhoden. Ergebn. med. Strahlenforsch. **1**, 443 (1925).
WARREN, S.: Effects of radiation on normal tissue. VIII. Effects on the gonads. Arch. Path. **35**, 121 (1943).

Damage due to Pressure, Torsion

ANGELL, J.C.: Torsion of the testicle. Lancet **1963 I**, 19.
LYON, R.P.: Torsion of the testicle in childhood. J. Amer. med. Ass. **178**, 702 (1961).
NOWAKOWSKI, H.: Bilateral testicular atrophy as a result of scrotal hematoma in the newborn. Acta endocr. (Kbh.) **18**, 506 (1955).
SCHÄRLI, A.: Probleme der Hodentorsion im Kindesalter. Schweiz. med Wschr. **1971**, 1096.

Germinal Aplasia

BOLLAG, W.: Cytostatica in der Schwangerschaft. Schweiz. med. Wschr. **1954**, 393.
CASTILLO, E.B. DEL, TRABUCCO, A., BALZE, F.A. DE LA: Syndrome produced by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells. J. clin. Endocr. **7**, 493 (1947).
CHAVES-CARBALLO, E., HAYLES, A.B.: Ullrich-Turner syndrome in the male: review of the literature and report of a case with lymphocytic (Hashimoto's) thyroidits. Proc. Mayo Clin. **41**, 843 (1967).
TRITSCH, H., SCHWARZ, G.: Germinalzell-Aplasie und Kleinwuchs. Dtsch. med. Wschr. **88**, 701 (1963).

Klinefelter's Syndrome

BECKER, K.L., ALBERT, A.: Urinary excretion of follicle-stimulating and luteinizing hormones. J. clin. Endocr. **25**, 962 (1965).

DAVIS, TH. E., CANFIELD, C. C. J., HERMAN, R. H., GOLES, D.: Thyroid function in patients with aspermiogenesis and testicular tubular sclerosis. New Engl. J. Med. **268**, 178 (1963).

DECOURT, J.: L'orchidodystrophie polygonosomique (syndrome de Klinefelter dit «vrai»). Sem. Hôp. Paris **38**, 1249 (1962).

— JAYLE, M. F., MICHARD, J. P., DROSDOWSKY, M.: Etude de la fonction Leydigienne du testicule dans 12 cas de syndrome de Klinefelter. Ann. Endocr. (Paris) **22**, 974 (1961).

FRANCHIMONT, P.: Radioimmunoassay of gonadotropines. Advanc. Biosciences **1**, 19 (1969).

GABRILOVE, J. L., NICOLIS, G. L., HAUSKNECHT, R. U.: Urinary testosterone, oestrogen production rate and urinary oestrogen in chromatin-positive Klinefelter's syndrome. Acta endocr. (Kbh.) **63**, 499 (1970).

GILBERT-DREYFUS, SEBAOUN, J., MALINSKY, M.: Histoire naturelle de la maladie de Klinefelter. Rev. franç. Endocr. **5**, 465 (1964).

GIORGIO, E. P., SOMMERVILLE, I. F.: Hormone assay in Klinefelter's syndrome. J. clin. Endocr. **23**, 197, 1963.

JACKSON, A. W., MULDAL, S., OCKEY, C. H., O'CONNOR, P. J.: Carcinoma of male breast in association with the Klinefelter syndrome. Brit. med. J. **1965 I**, 223.

JOHNSEN, S. G.: The mechanisms involved in testicular degeneration in man. Advance of a theory of the pathogenesis in "idiopathic" hypospermatogenesis. Acta endocr. (Kbh.), Suppl. **124**, 17 (1967).

KELLER, P. J.: Hormonal studies in endocrine disease. Germ. med. Mth. **13**, 389 (1968).

KLINEFELTER, H. F., REIFENSTEIN, E. C., ALBRIGHT, F.: Syndrome characterized by gynecomastia, spermatogenesis without A-Leydigism and increased excretion of follicle-stimulating hormone. J. clin. Endocr. **2**, 615 (1942).

KNORR, D., MÜRSET, G., PRADER, A., TOLKSDORF, M., WIEDEMANN, H. R.: Die Testosteronausscheidung beim chromatin positiven Klinefelter-Syndrom im Kindes- und Jugendalter. Acta endocr. (Kbh.) **56**, 65 (1967).

KOLLER, F., SIEGENTHALER, W.: Die Schilddrüsenfunktion beim Klinefelter-Syndrom. Schweiz. med. Wschr. **85**, 8 (1955).

LENZ, W., NOWAKOWSKI, H., PRADER, A., SCHIRREN, C.: Die Aetiologie des Klinefelter-Syndromes. Schweiz. med. Wschr. **89**, 727 (1959).

LIPSETT, M. B., DAVIS, T. E., WILSON, H., CANFIELD, C. J.: Testosterone production in chromatine-positive Klinefelter's syndrome. J. clin. Endocr. **25**, 1027 (1965).

LUBS, H. A.: Testicular size in Klinefelter's syndrome in men over 50. New Engl. J. Med. **267**, 326 (1962).

NIELSEN, J., JOHANSEN, K., YDE, H.: Frequency of diabetes mellitus in patients with Klinefelter's syndrome of different chromosome constitutions and the XYY syndrome. Plasma insulin and growth hormone level after a glucose load. J. clin. Endocr. **29**, 1062 (1969).

OVERZIER, C.: Das sog. echte Klinefelter-Syndrom. Internist (Berl.) **4**, 1 (1963).

PASI, A.: Häufigkeit des echten Klinefelter-Syndroms bei Autopsien. Schweiz. med. Wschr. **95**, 1580 (1965).

PAULSEN, A., GORDON, D. L., CARPENTER, W., GANDY, H. M., DRUCKER, W. D.: Klinefelter's syndrome and its variants: a hormonal and chromosomal study. Recent Progr. Hormone Res. **24**, 321 (1968).

PRADER, A.: Die Klinik der häufigsten chromosomalen Störungen. Helv. med. Acta **29**, 403 (1962).

— SCHNEIDER, J., ZÜBLIN, W., FRANCES, J. M., RÜEDI, K.: Die Häufigkeit des echten, chromatin-positiven Klinefelter-Syndroms und seine Beziehungen zum Schwachsinn. Schweiz. med. Wschr. **88**, 917 (1958).

SCHIBLER, D., KIND, H. P., BROOK, C. G. D., ZACHMANN, M., PRADER, A.: Growth and body proportions in chromatin positive Klinefelter's syndrome (in preparation, 1973).

SIEBENMANN, R., PRADER, A.: Das echte Klinefelter-Syndrom vor der Pubertät. Schweiz. med. Wschr. **88**, 607 (1958).

TANNER, J. M., PRADER, A., HABICH, H., FERGUSON-SMITH, M. A.: Genes on the y-Chromosome influencing rate of maturation in man. Lancet **1959 II**, 141.

WARBURG, E.: A fertile patient with Klinefelter's syndrome. Acta endocr. (Kbh.) **43**, 12 (1963).

ZUPPINGER, K., ENGEL, E., FORBES, A. P., MANTOOTH, L., CLAFFEY, J.: Klinefelter's syndrome, a clinical and cytogenetic study in twenty-four cases. Acta endocr. (Kbh.), Suppl. 113, ad vol. **54** (1967).

"False", Chromatin-Negative Klinefelter's Syndrome

BOWEN, P., LEE, C. S. N., MIGEON, C. J., KAPLAN, N. M., WHALLEY, T. J., MCKUSICK, V. A., REIFENSTEIN, E. C., JR.: Hereditary male pseudohermaphroditism with hypogonadism, hypospadia and gynecomastia. Ann. intern. Med. **62**, 252 (1965).

KAPLAN, E., SHWACHMAN, H., PERLMUTTER, A. D., RULE, A., KHAW, K.-T., HOLSCLAW, D. S.: Reproductive failure in males with cystic fibrosis. New Engl. J. Med. **279**, 65 (1968).

REIFENSTEIN, E. C., JR.: Hereditary familial hypogonadism (Abstract). Proc. Amer. Med. Clin. Res. **3**, 86 (1947).

XYY Trisomy, Other Chromosomal Deviations

CLEVELAND, W. W., ARIAS, D., SMITH, G. F.: Radioulnar synostosis, behavioral disturbance, and XYY chromosomes. J. Pediat. **74**, 103 (1969).

HIENZ, H. A.: YY-Syndrome forms. Lancet **1969 I**, 155.

ISMAIL, A. A. A., HARKNESS, R. A., KIRKHAM, K. E., LORAINE, J. A., WHATMORE, P. B., BRITTAIN, R. P.: Effect of abnormal sex chromosome complements on urinary testosterone levels. Lancet **1968 I**, 220.

JACOBS, P. A., BRUNTON, M., MELVILLE, M. M., BRITTAIN, R. P., MCCLEMONT, W. F.: Aggressive behavior, mental subnormality and the XYY male. Nature (Lond.) **208**, 1351 (1965).

NIELSEN, J., YDE, H., JOHANSEN, K.: Serum growth hormone level after oral glucose load, urinary excretion of pituitary gonadotrophin and 17-ketosteroids in XYY syndrome. Metabolism **18**, 993 (1969).

PARKER, C. E., MELNYK, J., FISH, C. H.: The XYY syndrome. Amer. J. Med. **47**, 801 (1969).

PFEIFFER, R. A., RIEMER, G., SCHNELLER, W.: Probleme der XYY-Konstitution. Med. Welt (Stuttg.) **20**, 75 (1969).

RUDD, B. T., GALAL, O. M., CASEY, M. D.: Testosterone excretion rates in normal males and males with an XYY complement. J. med. Génét. **5**, 286 (1968).

SERGOVICH, F. R.: Chromosome aberrations in 2159 consecutive newborn babies. New Engl. J. Med. **280**, 851 (1969).

The Male Climacteric

BÜRGI, H., HEDINGER, CHR.: Histologische Hodenveränderungen im hohen Alter. Schweiz. med. Wschr. **89**, 1236 (1959).

DRY, J.: La fonction gonadique du vieillard. In: Le testicule et l'hypogonadisme. masculin. Paris: L'expansion 1967.

Editorial: Is there a male climacteric? J. Amer. med. Ass. **155**, 1427 (1954).

FINKLE, A. L., MOYERS, T. G., TOBENKIN, M. I.: Sexual potency in aging males 1. Frequency of coitus among clinic patients. J. Amer. med. Ass. **170**, 1391 (1959).

HELLER, C. G., MYERS, G. B.: The male climacteric. Its symptomatology and treatment. J. Amer. med. Ass. **126**, 472 (1944).

NOWAKOWSKI, H., SCHMITT, H.: Die Hodenveränderungen beim alternden Mann und deren klinische Bedeutung. Schweiz. med. Wschr. **89**, 1204 (1959).
SPENCE, A.W.: The male climacteric, is it an entity? Brit. med. J. **1954 I**, 1355.

Secondary Testicular Insufficiency

Gonadotropin Failure: Tubular and Interstitial Insufficiency

BARTTER, F.C., SNIFFEN, R.C., SIMMONS, F.A., ALBRIGHT, F., HOWARD, R.T.: Effects of chorionic gonadotropin (A.P.L.) in male eunuchoidism with low follicle stimulating hormone J. clin. Endocr. **12**, 1532 (1952).
BIBEN, R.L., GORDON, G.S.: Familial hypogonadotropic eunuchoidism. J. clin. Endocr. **15**, 931 (1955).
COURVOISIER, B., MARTIN, E.: Syndrom hypothalamique et tuberculose atypique. Schweiz. med. Wschr. **1955**, 512.
DECOURT, J., DOUMIC, J.M.: Les retards pubertaires familiaux. Sem. Hôpt. Paris **34**, 2349 (1958).
— GENNES, J.L. DE, MICHARD, J.P., RAYMOND, J.P.: L'eunuchoidisme hypogonadotrophique, étude clinique et biologique. In: le testicule et l'hypogonadisme masculin. Paris: L'expansion 1967.
MARSHALL, J.C., HARSOULIS, P., ANDERSON, D.C., MCNEILLY, A.S., BESSER, G.M., HALL, R.: Isolated pituitary gonadotrophin deficiency: gonadotrophin secretion after synthetic luteinizing hormone and follicle stimulating hormone—releasing hormone. Brit. med. J. **1972**, **4**, 643.
MCCULLAGH, E.P., GOLD, A., MCKENDRY, J.B.T.: Alteration in testicular structures and function in organic disease of the pituitary. J. clin. Endocr. **10**, 871 (1950).
NOWAKOWSKI, H., LENZ, W.: Genetic aspects in male Hypogonadism. Recent Progr. Hormone Res. **17**, 53 (1961).
ROSEWATER, S., GWINUP, G., HAMWI, G.J.: Familial gynecomastia. Ann. intern. Med. **63**, 377 (1965).
SNIFFEN, R.C., HOWARD, R.P., SIMMONS, F.A.: The testis. IV. Idiopathic eunuchoidism with low FSH, testicular changes secondary to lesions in or near the pituitary and secondary to estrogen therapy. Arch. Path. **58**, 464 (1954).
VOLPÉ, R., METZLER, W.S., JOHNSTON, M.W.: Familial hypogonadotrophic eunuchoidism with cerebellar ataxia. J. clin. Endocr. **23**, 107 (1963).

Hypogonadotropic Hypogonadism with Anosmia

BARDIN, C.W., ROSS, G.T., RIFKIND, A.B., CARGILLE, C.M., LIPSETT, M.B.: Studies of the pituitary-Leydig cell axis in young men with hypogonadotropic hypogonadism and hyposmia: comparison with normal men, prepuberal boys, and hypopituitary patients. J. clin. Invest. **48**, 2046 (1969).
KALLMANN, F.J., SCHÖNFELD, W.A., BARRERA, S.E.: The genetic aspects of primary eunuchoidism. Amer. J. ment. Defic. **48**, 203 (1944).
MORSIER, G. DE: Etudes sur les dysraphies crânio-encéphaliques. I. Agénésie des lobes olfactifs et des commissures calleuses et antérieures. La dysplasie olfacto-génitale. Schweiz. Arch. Neurol. Psychiat. **74**, 309 (1954).
— GAUTHIER, G.: La dysplasie olfacto-génitale. Path. Biol. **11**, 1267 (1963).
NAFTOLIN, F., HARRIS, G.W.: Effect of purified luteinizing hormone releasing factor on normal and hypogondaotrophic anosmic men. Nature (Lond.) **232**, 496 (1971).
SAN JUAN, A.M., DE: Falta total de los nervios olfactorios con anosmia en un individuo en quien exista una atrofia congenita de los testiculos y miembro viril. Siglomedico (Madrid) p. 211 and 218, 1856.
SANTEN, R.J., PAULSEN, C.A.: Hypogonadotropic eunuchoidism. I. Clinical study of the mode of inheritance. J. clin. Endocr. **36**, 47 (1973).
— — Hypogonadotropic hypogonadism. II. Gonadal responsiveness to exogenons gonadotropins. J. clin. Endocr. **36**, 55 (1973).
TAGATZ, G., FIALKOW, PH, J., SMITH, D., SPADONI, L.: Hypogonadotrophic hypogonadism associated with anosmia in the female. New Engl. J. Med. **283**, 1326 (1970).
WEIDENREICH, F.: Über partiellen Riechlappendefekt und Eunuchoidismus beim Menschen. Z. Morph. Anthropol. **18**, 157 (1914).

Gonadotropin Failure in Hypothyreosis and in Undernutrition

BALZE, F.A. DE LA, ARRILLAGA, F., MANCINI, R.E., JANCHES, M., DAVIDSON, O.W., GURTMAN, A.I.: Male hypogonadism in hypothyroidism. J. clin. Endocr. **22**, 213 (1962).
JACOBS, E.C.: Effects of starvation on sex hormones in the male. J. clin. Endocr. **8**, 277 (1948).
LEDERER, J., ROGER, F., BATAILLE, J.P.: Le mécanisme de l'insuffisance génitale masculine dans l'hypothyroidie primaire. In: Le testicule et l'hypogonadisme masculin. Paris: l'Expansion 1967.
MANN, T.: Appraisal of endocrine testicular activity by chemical analysis of semen and male accessory secretions. Ciba Coll. Endocr. **16**, 233 (1967).
— ROWSON, L.E.A., SHORT, R.V., SKINNER, J.D.: The relationship between nutrition and androgenic activity in pubescent twin calves, and the effect of orchitis. J. Endocr. **38**, 455 (1967).
UEHLINGER, E.: Pathologische Anatomie der Hungerkrankheit und des Hungerödems. In: HOTTINGER, GSELL, UEHLINGER, SALZMANN, LABHART: Hungerkrankheit, Hungerödem, Hungertuberkulose. Basel: Schwabe 1948.

Adrogenital Syndrome

HEDINGER, CHR.: Beidseitige Hodentumoren und kongenitales, adrenogenitales Syndrom. Schweiz. Z. Path. **17**, 743 (1954).
NOWAKOWSKI, H., PÜSCHEL, L.: Das isosexuelle adrenogenitale Syndrom mit Nebennierenrindenhyperplasie beim erwachsenen Mann. Acta endocr. (Kbh.) **11**, 320 (1952).

Damage due to Estrogens

DUNN, C.W.: Stilboestrol induced testicular degeneration in hypersexual males. J. clin. Endocr. **1**, 643 (1941).

Cirrhosis of the Liver

BENNET, H.S., BAGGENSTOSS, A.H., BUTT, H.R.: The testis, breast and prostate of men who die of cirrhosis of the liver. Amer. J. clin. Path. **20**, 814 (1950).
GALVÃO-TELES, A., ANDERSON, D.C., BURKE, C.W., MARSHALL, J.C., CORKER, C.S., BOWN, R.L., CLARK, M.L.: Biologically active androgens and oestradiol in men with chronic liver disease. Lancet **1973 I**, 173.
GILBERT-DREYFUS, SEBAOUN, J., SCHAISON, G., DRY, F.: Le testicule dans l'hémochromatose idiopathique. In: Le testicule et l'hypogonadisme masculin. Paris: L'Expansion 1967.
HEDINGER, CHR.: Zur Pathologie der Hämochromatose. Hämochromatose als Syndrom. Helv. med. Acta **20** (Suppl.), 32 (1953).
LANCH, C.W., WILLIAMS, R.H.: Endocrine changes, associated with Laënnec's cirrhosis of liver. Amer. J. Med. **4**, 315 (1948).

Interstitial Insufficiency, "Fertile Eunuchs"

ALBERT, A.L., UNDERDAHL, O., GREENE, L.F., LORENZ, N.: Male hypogonadism. VII. The testis in partial gonadotropic failure during puberty (lack of LH only). Proc. Mayo Clin. **30**, 31 (1955).

FAIMAN, CH., HOFFMAN, D.L., RYAN, R.J., ALBERT, A.: "Fertile eunuch" syndrome: demonstration of isolated luteinizing hormone deficiency by a radioimmuno assay technique. Mayo Clin. Proc. **43**, 661 (1968).

MCCULLAGH, E.P., BECK, J.C., SCHAFFENBURG, C.A.: Syndrome of eunuchoidism with spermatogenesis, normal urinary FSH and low or normal ICSH ("Fertile eunuchs"). J. clin. Endocr. **13**, 489 (1953).

Antiandrogens

APOSTOLAKIS, M., TAMM, J., VOIGT, K.D.: The effects of HCG in Cyproterone-treated male subjects. In: TAMM, Testosterone. Stuttgart: Thieme 1968.

BORIS, A., SCOTT, J.W., DEMARTINO, L., COX, D.C.: Endocrine profile of a nonsteroidal antiandrogen N-(3,5-Dimethyl-4-isoxazolylmethyl) Pthalimide (DIMP). Acta endocr. (Kbh.) **72**, 604 (1973).

BREUER, H., HOFFMANN, W.: Effect of cyproterone on the biogenesis of testosterone and related compounds. In: TAMM, Testosterone. Stuttgart: Thieme 1968.

DORFMAN, R.I.: Antiandrogens. In: TAMM, Testosterone. Stuttgart: Thieme 1968.

DYMLING, J.-F., NILSSON, K.O., HÖKFELT, B.: The effect of soldactona (canrenoate-potassium) on plasma testosterone and androstenedione and urinary 17-ketosteroids and 17-hydroxycorticosteroids. Acta endocr. (Kbh.) **70**, 104 (1972).

FANG, S., ANDERSON, K.M., LIAO, S.: Receptor proteins for androgens. On the role of specific proteins in selective retention of 17β-hydroxy-5α-androstan-3-one by rat ventral prostate *in vivo* and *in vitro*. J. biol. Chem. **244**, 6584 (1969).

LERNER, L.J.: Hormone antagonists: inhibitors of specific activities of Estrogen and Androgen. Recent Progr. Hormone Res. **20**, 435 (1964).

NEUMANN, F., ELGER, W., BERSWORDT-WALLRABE, R. VON: Intersexualität männlicher Foeten und Hemmung androgenabhängiger Funktionen bei erwachsenen Tieren durch Testosteronblocker. Dtsch. med. Wschr. **92**, 360 (1967).

— — STEINBECK, H., BERSWORDT-WALLRABE, R. VON: Antiandrogene. In: Testosteron — Struma. 13. Symp. dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.

— STEINBECK, H.: Antiandrogene. Tierexperimentelle Grundlagen und klinische Anwendungsmöglichkeiten. Internist (Berl.) **12**, 198 (1971).

OTT, F., HOFFE, H.: Beeinflussung von Libido, Potenz und Hodenfunktion durch Antiandrogene. Schweiz. med. Wschr. **98**, 1812 (1968).

— — HODEL, H.: Über die Erholung der Spermiogenese nach Behandlung mit Cyproteronazetat. Schweiz. med. Wschr. **102**, 1124 (1972).

SHERINS, R.J., GANDY, H.M., THORSLUND, T.W., PAULSEN, C.A.: Pituitary and testicular function studies. I. Experience with a new gonadal inhibitor, 17α-pregn-4-en-20-yno-(2, 3-d) isoxazol-17-ol (danazol). J. clin. Endocr. **32**, 522 (1971).

TAMM, J.: Testosterone. Proceedings of the workshop conference. Tremsbüttel 1967. Stuttgart: Thieme 1968.

VOIGT, K.D., APOSTOLAKIS, M., KLOSTERHALFEN, H.: The influence of Cyproterone treatment on the excretion of steroids in male patients. In: TAMM, Testosterone. Stuttgart: Thieme 1968.

Therapy

AAKVAAG, A., VOGT, J.H.: Plasma testosterone values in different forms of testosterone treatment. Acta endocr. (Kbh.) **60**, 537 (1969).

BARTTER, F.C., SNIFFEN, R.C., SIMMONDS, F.A., ALBRIGHT, F., HOWARD, R.T.: Effects of chorionic gonadotropin in male eunuchoidism with low follicle stimulating hormone. J. clin. Endocr. **12**, 1532 (1952).

DICZFALUSY, E., CASSMER, O.: Urinary 17-ketosteroids following administration of long acting testosterone esters. J. clin. Endocr. **21**, 271 (1961).

JOHNSEN, S.G.: The management of male hypogonadism. Acta endocr. (Kbh.), Suppl. 66 zu Bd. **40** (1962).

KUPPERMANN, H.S., ARONSON, S.G., GAGLIANI, J., PARSONETT, M., ROBERTS, M., SILVER, P., POSTIGLIONE, R.: The value of various laboratory procedures in the comparative study of the duration of action of androgens. Acta endocr. (Kbh.) **16**, 101 (1954).

LUDVIK, W.: Grundzüge moderner Androgenbehandlung. Urologe **9**, 41 (1970).

PETRY, R., RAUSCH-STROOMANN, J.-G., HIENZ, H.A., SENGE, TH., MAUSS, J.: Androgen treatment without inhibiting effect on hypophysis and male gonads. Acta endocr. (Kbh.) **59**, 497 (1968).

— RAUSCH-STROOMANN, J.G., SCHMIDT-ELMENDORFF, H.: Erfahrungen mit dem Androgen-Präparat Mesterolon. In: Das Testosteron — Die Struma. 13. Symp. dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.

REISS, M., HILLMAN, J., DAVIS, R.H.: Effect of prolonged treatment with human chorionic gonadotropin on the endogenous steroid hormone metabolism of physically and mentally retarded boys. J. clin. Endocr. **23**, 11 (1963).

ZACHMANN, M., PRADER, A.: Anabolic and androgenic effect of testosterone in sexually immature boys and its dependency on growth hormone. J. clin. Endocr. **30**, 85 (1970).

Cryptorchidism

Leading article: Undescended testis. Lancet **1970I**, 248.

BERGSTRAND, C.G., QVIST, O.: Treatment of undescended testis with human chorionic gonadotrophin. Acta endocr. (Kbh.) **37**, 231 (1961).

BIERICH, J.R.: Moderne Gesichtspunkte zum Problem des Hodenhochstandes. Internist (Berl.) **8**, 42 (1967).

BONGIOVANNI, A.M.: Diagnosis and treatment: The undescended testicle. Pediatrics **36**, 786 (1965).

CAMPBELL, H.E.: The incidence of malignant growth of undescended testes: Reply and evaluation. J. Urol. (Baltimore) **81**, 663 (1959).

CHARNY, C.W., WOLGIN, W.: Cryptorchism. New York: Paul B. Hoeber Inc. 1957.

COUR-PALAIS, I.J.: Spontaneous descent of the testicle. Lancet **1966I**, 1403.

GILBERT, J.B.: Studies in malignant testis tumors. Tumors developing after orchidopexy: Report of two cases and review of sixty-three. J. Urol. (Baltimore) **46**, 740 (1941).

GROSS, R.E., JEWETT, T.C.: Surgical experience from 122 operations of undescended testes. J. Amer. med. Ass. **160**, 634 (1956).

HAMILTON, W., GRANT, J.C., WILSON, R.R.: *In vitro* enzymic activity of human undescended testes. In: Errors of phenylalanine thyroxine and testosterone metabolism, p. 105. Edinburgh and London: E. & S. Livingstone 1970.

HECKER, W.CH., HIENZ, H.A., DAUM, R., HOLLMANN, G.: Zum Kryptorchismusproblem. Vergleichende morphologische und statistische Untersuchungen an beiden Hoden bei ein- und beidseitiger Dystopie. Dtsch. med. Wschr. **92**, 786 (1967).

HEDINGER, CHR., HÖSLI, P.: Welches ist der optimale Zeitpunkt der Behandlung. Schweiz. med. Wschr. **101** (1971) (in press).
HORTLING, H., CHAPELLE, A. DE LA, JOHANSSON, C.-J., NIEMI, M., SULAMAA, M.: An endocrinologic follow-up study of operated cases of cryptorchism. J. clin. Endocr. **27**, 120 (1967).
HUBER, R., WEBER, E., HEDINGER, CHR.: Struktur intratubulärer Körperchen (sog. Ringtubuli) des kindlichen Hodens. Virchows Arch. Abt. A Path. Anat. **344**, 40 (1968).
KLEIN, D., FERRIER, P., AMMANN, F.: La génétique de l'ectopie testiculaire. Path. Biol. **11**, 1214 (1963).
KNORR, D.: Diagnose und Therapie der Deszensusstörungen des Hodens. Päd. Praxis **9**, 299 (1970).
LARON, Z., ZILKA, E.: Compensatory hypertrophy of testicle in unilateral cryptorchidism. J. clin. Endocr. **29**, 1409 (1969).
SALLE, B., HEDINGER, CHR., NICOLE, R.: Significance of testicular biopsies in cryptorchidism in children. Acta endocr. (Kbh.) **58**, 67 (1968).
SCHEIBLI, P.: Häufigkeit von sogenannten Ringtubuli mit intratubulären Körperchen in normal descendierten und fehlgelagerten Knabenhoden. Virchows Arch. Abt. A Path. Anat. **344**, 249 (1968).
SCORER, C.G.: The descent of the testis. Arch. Dis. Childh. **39**, 605 (1964).
VILLUMSEN, A.L., ZACHAU-CHRISTIANSEN, B.: Spontaneous alterations in position of the testes. Arch. Dis. Childh. **41**, 198 (1966).

Sterility

BEHRMAN, S.J., KISTNER, R.W.: Progress in infertility. Boston: Little, Brown & Co. 1968.
BISHOP, P.M.F.: Endocrine aspects of infertility in men (Transient infertility). J. Amer. med. Ass. **163**, 534 (1953).
BORELLI, S., DOEPFNER, R., HEINKE, E.: Fertilitätsstörungen beim Manne. In: Handbuch der Haut- und Geschlechtskrankheiten, vol. 6/3, H. SCHUERMANN and R. DOEPFNER, eds. Berlin-Göttingen-Heidelberg: Springer 1960.
FARRIS, E.J.: Human fertility. The authors press. New York: Whiteplans 1950.
HOTCHKISS, R.S.: Fertility in men. Philadelphia: J.B. Lipincott 1944.
JOËL, CH.A. (ed.): Fertility disturbances in men and women. Basel-München-New York: Karger 1971.
KIMMIG, J., STEENO, O., SCHIRREN, C.: Ergebnisse der modernen biochemischen Forschungen auf dem Gebiete der Andrologie. Internist (Berl.) **8**, 25 (1967).
MANN, T.: Fructose and fructolysis in semen in relation to fertility. Lancet **1948 I**, 446.
— Advances in male reproductive physiology. Fertil. Steril. **23**, 699 (1972).
— Biochemistry of semen and of the male reproductive tract. London: Methuen 1964.
— The science of reproduction. Nature **224**, 649 (1969).
MCLANE, CH.M.: Symposium on infertility. Clin. Obstet. Gynec. **8**, (1965).
SCHIRREN, C.: Fertilitätsstörungen beim Manne. Enke: Stuttgart 1961.
— Neue Ergebnisse der Andrologie. Berlin-Heidelberg-New York: Springer 1965.
— Klinik der Andrologie. Internist (Berl.) **8**, 2 (1967).
— (ed.): Fortschritte in der Andrologie, Vol. 1: Morphological aspects of andrology by A.F. HOLSTEIN, E. HORSTMANN. Berlin: Grosse 1970.
TYLER, E.T.: Physiological and clinical aspects of conception. J. Amer. med. Ass. **153**, 1351 (1953).
VASTERLING, H.W.: Praktische Spermatologie: Stuttgart: Thieme 1960.
WOLSTENHOLME, G.E.W. (ed.): Mammalian germ cells. Ciba Foundation Symp. London: Churchill 1953.

Etiology and Pathogenesis of Sterility

AUSTIN, C.R.: Sperm capacitation—biological significance in various species. Advanc. Biosciences **4**, 5 (1970).
BEDFORD, J.M.: Morphological aspects of sperm capacitation in mammals. Advanc. Biosciences **4**, 35 (1970).
BEHRMAN, S.J., BÜTTNER-JANUSCH, J., HEGLAR, R., GERSKOWITZ, H., TEW, W.L.: ABO(H) blood incompatibility as a cause of infertility: a new concept. Amer. J. Obstet. Gynec. **79**, 847 (1960).
— NAKAYAMA, M.: Antitestis antibody: its inhibition of pregnancy Fertil. Steril. **16**, 37 (1965).
CHANG, M.C.: Hormonal regulation of sperm capacitation. Advanc. Biosciences **4**, 13 (1970).
CHARNY, CH.W.: Effect of varicocele on fertility, results of varicocelectomy. Fertil. Steril. **13**, 47 (1962).
DOEPFNER, R.: Die klinische Bedeutung der absoluten, der partiellen (einseitigen) und der relativen Polyspermie der männlichen Infertilität. Arch. klin. exp. Derm. **215**, 246 (1962).
DUKELOW, W.R.: Bioassay technics related to sperm capacitation. Acta endocr. (Kbh.) **66**, 503 (1971).
DUNCAN, G.W., ERICSSON, R.I., ZIMBELMAN, R.G. (eds.): Capacitation of spermatozoa and endocrine control of spermatogenesis. J. Reprod. Fertil., Suppl. **2** (1967).
EDWARDS, R.G.: Immunology of conception and pregnancy. Brit. med. Bull. **26**, 72 (1970).
ERB, H., LOOSLI, R., SCHÄRER, K., WENNER, R.: L'influence des médicaments sur la fertilité masculine. Méd. Hyg. (Genève) **22**, 833 (1964).
FAIRLEY, K.F., BARRIE, J.U., JOHNSON, W.: Sterility and testicular atrophy related to cyclophosphamide therapy. Lancet **1972 I**, 568.
FJÄLLBRANT, B.: Immunoagglutination of sperm in cases of sterility. Acta obstet. gynec. scand. **44**, 474 (1965).
HOTCHKISS, R.S.: Etiology and diagnosis in the treatment of infertility in men. Springfield: Ch.C. Thomas 1952.
JOEL, C.A.: Zur Ätiologie des habituellen Abortes unter Berücksichtigung des männlichen Faktors. Gynaecologia (Basel) **154**, 257 (1962).
JOHNSEN, S.G.: The mechanisms involved in testicular degeneration in man. Advance of a theory of the pathogenesis in "idiopathic" hypospermatogenesis. Acta endocr. (Kbh.), Suppl. 124, to vol. **56**, 17 (1967).
— Studies on the pituitary-testicular axis in male hypogonadism, particularly in infertile men with cryptogenetic hypospermatogenesis. In: Gonadotropins, SAXENA *et al.*, eds. New York: Wiley-Interscience 1972.
KATSH, S., KATSH, G.F.: Antigenicity of spermatozoa. Fertil. Steril. **12**, 522 (1961).
KIESSLING, W., HUHNSTOCK, K.: Aspermatismus nach peroralen Gaben von Guanethidin. Klin. Wschr. **41**, 948 (1963).
KJESSLER, B.: Karyotype, meiosis and spermatogenesis in a sample of men attending an infertility clinic. Basel, New York: Karger 1966.
LEUCHTENBERGER, C., WEIR, D.R., SCHRADER, F., LEUCHTENBERGER, R.: Decreased amounts of desoxyribose nucleid acid (DNA) in male germ cells as a possible cause of human male infertility. Acta genet. (Basel) **6**, 272 (1957).
LEVENE, H., ROSENFIELD, R.E.: ABO-Incompatibility. In: A.G. STEINBERG, Progress in medical genetics. New York: Grune & Stratton 1961.
MACHER, E.: Die ABO-Inkompatibilität als fertilitätsdiagnostisches und therapeutisches Problem. Arch. klin. exp. Derm. **219**, 933 (1964).
MACLEOD, J., GOLD, R.Z.: The male factor in fertility and infertility. IX. Semen quality in relation to accidents of pregnancy. Fertil. and Steril. **8**, 36 (1957).

MacLeod, J., Gold, R. Z., MacLane, Ch. M.: Correlation of the male and female factors in human infertility. Fertil. Steril. **6**, 112 (1955).
Mancini, R. E.: Allergic aspermatogenesis experimentally induced in men and animals. In: Injury, inflammation and immunity, Thomas. Uhr and Grant, eds. Baltimore: Williams & Wilkins 1964.
— Andrada, J. A., Saraceni, D., Bachmann, A. E., Lavieri, J. C., Nemirovsky, M.: Immunological and testicular response in man sensitized with human testicular homogenate. J. clin. Endocr. **25**, 859 (1965).
Mann, T.: Effect of pharmacological agents on male sexual functions. J. Reprod. Fertil., Suppl. **4**, 101 (1968).
Meyhöfer, W.: Mikrospektrophotometrische Messungen des Nucleinsäuregehaltes von Spermien fertiler und infertiler Männer. Arch. klin. exp. Derm. **216**, 556 (1963).
Nakabajashi, N. T., Tyler, E. T., Tyler, A.: Immunologic aspects of human infertility. Fertil. Steril. **12**, 544 (1961).
Nelson, W. O.: Etiologic factors in spermatogenic arrest. J. clin. Endocr. **14**, 799 (1954).
Pikò, L.: Immunological phenomena in the reproductive process. In: Fertility and sterility. Proc. 5th world Congr. Amsterdam: Excerpta med. Foundation 1967.
Rümke, P.: Autospermagglutinins: a cause of infertility in men. Ann. N. Y. Acad. Sci. **124**, 696 (1965).
Rümke, Ph.: Autoantikörperbildung gegen Spermatozoen infolge Extravasation von Spermatozoen ins Interstitium der Epididymis älterer Männer. Schweiz. med. Wschr. **101**, 1439 (1971).
Scott, L. S.: Varicocele ligation in subfertile men. In: Fertility and sterility. Proc. 5th world Congr., Westin and Wiqrist, eds. Amsterdam; Excerpta med. Foundation 1967.
— Young, D.: Varicocele: a study of its effects on human spermatogenesis and of the results produced by spermatic vein ligation. Fertil. Steril. **13**, 325 (1962).
Sobbe, A., Haferkamp, O., Doepfner, R.: Serologische und immunbiologische Untersuchungen an Sperma und Seren von Männern steriler Ehen. Dtsch. med. Wschr. **91**, 1234 (1966).
Williams, W. L., Robertson, R. T., Dukelow, W. R.: Decapacitation factor and capacitation. Advanc. Biosciences **4**, 61 (1970).

Induced Reversible Sterility in the Male

Chang, M. C.: Capacitation of rabbit spermatozoa in utero, with special reference to reproductive phases of female. Endocrinology **63**, 619 (1958).
Jackson, H.: Antifertility compounds in the male and female. Springfield: C. C. Thomas 1966.
— Antispermatogenic agents. Brit. med. Bull. **26**, 79 (1970).
Mancini, R. E., Andrada, J. A., Saraceni, D., Bachmann, A. E., Lavieri, J. C., Nemirovsky, M.: Immunological and testicular response in man sensitized with human testicular homogenate. J. clin. Endocr. **25**, 859 (1965).
Mann, T.: Effects of pharmacological agents on male sexual functions. J. Reprod. Fertil., Suppl. **4**, 101 (1968).
Patanelli, D. J., Nelson, W. O.: A quantitative study of inhibition and recovery of spermatogenesis. Recent Progr. Hormone Res. **20**, 491 (1964).
Petry, R., Rausch-Stroomann, J.-G.: Gegenwärtiger Stand der Fertilitätshemmung beim Mann. Dtsch. med. Wschr. **95**, 855 (1970).
Pincus, G.: The control of fertility. New York and London: Academic Press 1965.
Segal, S. J.: Immunology and infertility. Fertil. Steril. **12**, 520 (1961).
— Research in fertility regulation. New Engl. J. med. **279**, 364 (1968).
Sherins, R. J., Gandy, H. M., Thorslund, T. W., Paulsen, C. A.: Pituitary and testicular function studies. I. Experience with a new gonadal inhibitor, 17α-pregn-4-en-20-yno-(2,3-d) isoxazol-17-ol (danazol). J. clin. Endocr. **32**, 522 (1971).
Sobbe, A., Haferkamp, O., Doepfner, R.: Serologische und immunohistologische Untersuchungen an Sperma und Seren von Männern steriler Ehen. Dtsch. med. Wschr. **91**, 1234 (1966).
Weil, A. J.: Antigens of the adnexal glands of the male genital tract. Fertil. Steril. **12**, 538 (1961).
Wyper, J. F. B.: Pregnancy after primary infertility investigation. Brit. med. J. **1962 I**, 273.

Therapy

Bayle, H.: Stérilité masculine: résultats opératoires du traitement des azoospermies excrétoires. Statistique personnelle de 248 anastomoses épididymodéférentielles. Presse méd. **68**, 760 (1960).
— Traitement des azoospermies excrétoires par l'anastomose épididymo-déférentielle latéro-latérale. Méd. Hyg. (Genève) **27**, 1365 (1969).
Bettendorf, G.: Beziehungen zwischen Andrologie und Gynäkologie. Internist (Berl.) **8**, 38 (1967).
Blanchard, J. R.: Resultate von 837 Samenuntersuchungen und Diskussion von Therapieresultaten. Schweiz. med. Wschr. **97**, 163 (1967).
Boccabella, A. V.: Reorientation and restoration of spermatogenesis with testosterone propionate and other hormones after long-term post-hypophysectomy regression period. Endocrinology **72**, 787 (1963).
Brown, J. S., Dubin, L., Hotchkiss, R. S.: The varicocele as related to fertility. Fertil. Steril. **18**, 46 (1967).
Charny, Ch. W.: The use of androgens for human spermatogenesis. Fertil. Steril. **10**, 557 (1959).
Davis, M. E.: Management of infertility. J. Amer. med. Ass. **201**, 1030 (1967).
Donini, P., Puzzuoli, D., Montezemolo, R.: Purification of gonadotrophin from human menopausal urine. Acta endocr. (Kbh.) **45**, 321 (1964).
Farris, E. J., Murphy, D. P.: The characteristics of the 2 parts of the partitioned ejaculate and the advantages of its use for intrauterine insemination. Fertil. Steril. **11**, 465 (1960).
Finegold, W. J.: Artificial insemination. Springfield: Thomas 1964.
Gemzell, C., Kjessler, B.: Treatment of infertility after partial hypophysectomy with human pituitary gonadotrophins. Lancet **1964 I**, 644.
Giesen, D.: Die künstliche Insemination als ethisches und rechtliches Problem. Bielefeld: Giesking 1962.
Glatthaar, E.: Probleme der heterologen artifiziellen Insemination. Schweiz. med. Wschr. **101**, 489 (1971).
Guttmacher, A. F.: The role of artificial insemination in the treatment of sterility. Obstet. gynec. Surg. **15**, 767 (1960).
Hanley, H. G., Harrison, R. G.: The nature and surgical treatment of varicocele. Brit. J. Surg. **50**, 64 (1962/63).
Heckel, N. J., Rosso, W. A., Kestel, L.: Spermatogenic rebound phenomenon after administration of testosterone propionate. J. clin. Endocr. **11**, 235 (1951).
Heller, C. G., Moore, D. J.: Dichotomy between total gonadotrophins (HPG) and ICSH excretion produced by Clomiphen. Clin. Res. **11**, 111 (1963).
Horne, H. W., Jr.: Coital positioning in infertility. Fertil. Steril. **12**, 319 (1961).
Husslein, H., Eisenhut, L.: Die artifizielle Insemination. Wien. med. Wschr. **116**, 325 (1966).
Joel, C. A.: The spermiogenetic rebound phenomenon and its clinical significance. Fertil. Steril. **11**, 384 (1960).

JOHNSEN, S.G.: A study of human testicular function by the use of human menopausal gonadotrophin and of human chorionic gonadotrophin in male hypogonadotrophic eunuchoidism and infantilism. Acta endocr. (Kbh.) **53**, 315 (1966).

JUNGCK, E.C., ROY, S., GREENBLATT, R.G., MAHESH, V.P.: Effect of Clomiphen citrate on spermatogenesis in the human. Fertil. Steril. **15**, 40 (1964).

KLOSTERHALFEN, H.: Beziehungen zwischen Andrologie und Urologie. Internist (Berl.) **8**, 34 (1967).

LUNENFELD, B.: Gonadotrophin therapy. Studies in male subjects. In: E.T. BELL and J.A. LORAINE: Recent research on gonadotrophic hormones. Edinburgh: Livingstone 1967.

— MOOR, A., MANI, M.: Treatment of male infertility. Fertil. Steril. **18**, 581 (1967).

LYTTON, B., KASE, N.: Effects of human menopausal gonadotrophin on a eunuchoidal male. New Engl. J. Med. **274**, 1061 (1966).

MACLEOD, J.: Seminal cytology in the presence of varicocele. Fertil. Steril. **16**, 735 (1965).

— Male infertility. Advances Obstet. and Gynec., vol. 1, MARCUS, ST.L. u. MARCUS, C.C., eds. Baltimore Williams & Wilkins 1967.

— PAZIANOS, A., RAY, B.: Restoration of human spermatogenesis by menopausal gonadotrophins. Lancet **1964 I**, 1196.

— — — The restoration of human spermatogenesis and of the reproductive tract with urinary gonadotropins following hypophysectomy. Fertil. Steril. **17**, 7 (1966).

MADDOCK, W.O., NELSON, W.O.: The effects of chorionic gonadotropin in adult men. J. clin. Endocr. **12**, 985 (1952).

MARTIN, F.I.R.: The stimulation and prolonged maintenance of spermatogenesis by human pituitary gonadotrophins in a patient with hypogonadotrophic hypogonadism. J. Endocr. **38**, 431 (1967).

MASTROIANNI, L., JR., LABERGE, J.L., ROCK, J.: Appraisal of the efficacy of artificial insemination with husband's sperm and evaluation of insemination technics. Fertil. Steril. **8**, 260 (1957).

MELLINGER, R.C., TOMPSON, K.J.: The effect of Clomiphen citrate in male infertility. Fertil. and Steril. **17**, 94 (1966).

MROUEH, A., LYTTON, B., KASE, N.: Effects of human chorionic gonadotropin and human menopausal gonadotropin (Pergonal) in males with oligospermia. J. clin. Endocr. **27**, 53 (1967).

MURPHY, D.P., TORRANO, E.F.: Male fertility in 3620 childless couples. Fertil. and Steril. **16**, 337 (1965).

O'CONNOR, C.J.: Mechanical aspects and surgical management of sterility in men. J. Amer. med. Ass. **153**, 532 (1953).

PERLOFF, W.H., STEINBERGER, E., SHERMAN, J.K.: Conception with human spermatozoa frozen by nitrogen vapor technique. Fertil. and Steril. **15**, 501 (1964).

POLISHUK, W.Z., PALTI, Z., LAUFER, A.: Treatment of defective spermatogenesis with human gonadotropins. Fertil. Steril. **18**, 127 (1967).

ROOS, P.: Human follicle-stimulating hormone. Uppsala: Almqvist & Wiksell 1967.

RUBIN, A.: The relationship of type of seminal deficiency to difficulty of conception based on experience in five hundred inseminations. Fertil. Steril. **12**, 581, 1962.

RUSSEL, J.K.: Varicocele, age and fertility. Lancet **1957 II**, 222.

SCHELLEN, A.M.C.M.: Artificial insemination in the human. Amsterdam: Elsevier 1957.

SCOTT, L.S., YOUNG, D.: Varicocele, a study of its effects on human spermatogenesis, and of the results produced by spermatic vein ligation. Fertil. Steril. **13**, 325 (1962).

SHERMAN, J.K.: Research on frozen human semen: past, present and future. Fertil. Steril. **15**, 485 (1965).

STAEHLER, W.: Die Indikation zu Refertilitätsoperationen. Dtsch. med. Wschr. **90**, 1180 (1965).

WYSS, H.I.: Diagnose und Therapie der männlichen Sterilität. Schweiz. med. Wschr. **97**, 1364 (1967).

ZANARTU, J., HAMBLEN, E.C.: Effect of oligospermia in insemination and fecundation in fertile women. Fertil. Steril. **11**, 248 (1960).

Examinations of the Semen

DOEPFNER, R.: Die Bedeutung des Spermiogramms beim primären Hodenschaden unter Berücksichtigung des einseitig deszendierten Hodens. Arch. klin. exp. Derm. **219**, 884 (1964).

ENGLE, E.T., SOUTHAM, A.: Evaluation of the barren marriage. Minimal procedures. The American Society for the Study of Sterility. Springfield, Mass. (USA) 1944.

FARRIS, E.J.: Human fertility. New York: The authors press. 1950.

HOTCHKISS, R.S.: Etiology and diagnosis in the treatment of infertility in men. Springfield: Ch.C. Thomas 1952.

JOEL, C.A.: Studien am menschlichen Sperma. 2nd ed. Basel: Benno Schwabe & Co. 1953.

MACLEOD, J.: The semen examination. Clin. Obstet. Gynec. **8**, 115 (1965).

MANN, T.: Biochemistry of semen and of the male reproductive tract. London: Methuen 1964.

— Appraisal of endocrine testicular activity by chemical analysis of semen and male accessory secretions. Ciba Coll. Endocr. **16**, 233 (1967).

— ROTTENBERG, D.A.: The carbohydrate of human semen. J. Endocr. **34**, 257 (1966).

— Chemical analysis of semen as a diagnostic aid in reproductive failure. Proc. VI. World Congr. on Fertility Sterility, p. 251, The Israel Academy of Sciences and Humanities, Jerusalem 1970.

NELSON, W.O.: Spermatogenesis in men with blocked or absent efferent ducts. In: T.E. ENGLE, Studies on testis and ovary, eggs and sperm. Springfield: Ch.C. Thomas 1952.

NOWAKOWSKI, H., SCHIRREN, C.: Sperma fructose und Leydigzellenfunktion beim Manne. Klin. Wschr. **34**, 19 (1956).

SILLO-SEIDL, G.: Die Bewegungslehre der menschlichen Samenfäden. Fortschritte auf dem Gebiete der Gynäkologie Basel: Karger 1963.

VASTERLING, H.W.: Praktische Spermatologie. Stuttgart: Thieme 1960.

Hormonal Treatment of Impotence, Satyrism, Perversion

COOPER, A.J., ISMAIL, A.A.A., SMITH, C.G., LORAINE, J.A.: Androgen function in "psychogenic" and constitutional types of impotence. Brit. med. J. **1970 II**, 17.

DUNN, C.W.: Stilboestrol induced testicular degeneration in hypersexual males. J. clin. Endocr. **1**, 643 (1941).

FAERMAN, I., VILAR, O., RIVAROLA, M.A., ROSNER, J.M., JADZINSKY, M.N., FOX, D., LLORET, A.P., BERNSTEIN-HAHN, L., SARACENI, D.: Impotence and Diabetes. Studies of androgenic function in diabetic impotent males. Diabetes **21**, 23 (1972).

KRAUSE, W.F.J.: Behandlung funktioneller Sexualstörungen (insbes. der Ejaculatio praecox). Erfahrungen mit Chlormadinonacetat. Münch. med. Wschr. **112**, 966 (1970).

LASCHET, U., LASCHET, L.: Die Behandlung der pathologisch gesteigerten und abartigen Sexualität des Mannes mit dem Antiandrogen Cyproteronacetat. In: Testosteron—Struma. 13. Symp. d. Dtsch. Ges. Endocr. Berlin-Heidelberg-New York: Springer 1968.

OTT, F., HOFFE, H.: Beeinflussung von Libido, Potenz und Hodenfunktion durch Antiandrogene. Schweiz. med. Wschr. **98**, 1812 (1968).

Gynecomastia

BECKER, K. L., WINNACKER, J. L., MATTHEWS, M. J., HIGGINS, G. A.: Gynecomastia and Hyperthyroidism. An endocrine and histological investigation. J. clin. Endocr. **28**, 277 (1968).

BRONSTEIN, I.-P., SHADAKSHARAPPA, K. S.: Gynecomastia. In: SOSKIN, Progress in clin. endocrinol. New York: Grune & Stratton 1950.

CAPLAN, R. M.: Gynecomastia from a non-estrogenic antiandrogen. J. clin. Endocr. **27**, 1348 (1967).

DECOURT, J., JAYLE, M. F., MASSIN, J. P.: Étude de 49 cas de gynécomasties apparement isolées de l'adolescence. Sem. Hôp. Paris **38**, 1266 (1962).

FREEMAN, R. M., LAWTON, R. L., FEARING, M. O.: Gynecomastia: an endocrinologic complication of hemodialysis. Ann. intern. Med. **62**, 67 (1968).

HALL, P. F.: Gynaecomastia. Sidney: Austral. med. publish. Co. 1959.

HAURI, D., ZINGG, E.: Mamillenbestrahlung bei Hormonbehandlung des Prostatakarzinoms. Schweiz. med. Wschr. **101**, 571 (1971).

JULL, J. W., BONSER, G. M., DOSSETT, J. A.: Hormone excretion studies of males with gynecomastia. Brit. med. J. **1964 II**, 797.

— DOSSETT, J. A.: Hormone excretion studies of gynecomastia of puberty. Brit. med. J. **1964 II**, 795.

LJUNGBERG, T.: Hereditary gynecomastia. Acta med. scand. **168**, 371 (1960).

MOLINA, C., ABERKANE, B. P.: Les gynécomasties des tuberculeux pulmonaires. Sem. Hôp. Paris **36**, 194 (1960).

NICOLIS, G. L., MODLINGER, R. S., GABRILOVE, J. L.: A study of the histopathology of human gynecomastia. J. clin. Endocr. **32**, 173 (1971).

NYDICK, M., BUSTOS, J., DALE, J. H., CHESTER, P., RAWSON, R. W.: Gynecomastia in adolescent boys. J. Amer. med. Ass. **178**, 449 (1961).

PETERS, H. J., SIEBER, W. K., DAVIS, N.: Familial gynecomastia associated with genital abnormalities: report of family. J. clin. Endocr. **15**, 182 (1955).

PICARD, J. D.: Examen radiologique du sein chez l'homme. Ann. Radiol. **8**, 477 (1965).

RITSCHEL, E., SCHULTZE-JENA, B. S.: Über das vermehrte Auftreten der Fibrosis mammae virilis (sog. Gynäkomastie) in der Nachkriegszeit. Frankfurt. Z. Path. **61**, 476 (1949/50).

ROSEWATER, S., GWINUP, G., HAMWI, G. J.: Familial gynecomastia. Ann. intern. Med. **63**, 377 (1965).

RUPP, J., CANTAROW, A., RAKOFF, A. E., PASCHKIS, K. E.: Hormone excretion in liver disease and in gynecomastia. J. clin. Endocr. **11**, 688 (1951).

SAWIN, C. T., LONGCOPE, C., SCHMITT, G. W., RYAN, R.: Blood levels of gonadotropins and gonadal hormones in gynecomastia associated with chronic hemodialysis. J. clin. Endocr. **36**, 988 (1973).

SCHMITT, G. W., SHEHADEH, J., SAVIN, C. G.: Transient gynecomastia in chronic renal failure during chronic intermittent hemodialysis. Ann. intern. med. **69**, 73 (1968).

SHUSTER, S., BROWN, J. B.: Gynaecomastia and urinary oestrogens in patients with generalised skin disease. Lancet **1962 II**, 1358.

SPANKUS, W. H., GRANT, R. S.: Gynecomastia, J. clin. Endocr. **7**, 586 (1947).

STEINER, H., DAHLBÄCK, K. O., WALDENSTRÖM, J.: Ectopic growth hormone production and osteoarthropathy in bronchial tumor. Lancet **1968 I**, 783.

STOKES, J. F.: Unexpected gynecomastia. Lancet **1962 II**, 911.

TURKINGTON, R. W.: Serum prolactin levels in patients with gynecomastia. J. clin. Endocr. **34**, 62 (1972).

WALLACH, E. E., GARCIA, C. R.: Familial gynecomastia without hypogonadism: a report of three cases in one family. J. clin. Endocr. **22**, 1201 (1962).

WEBSTER, J. P.: Mastectomy for gynecomastia through semicircular intraareolar incision. Ann. Surg. **124**, 557 (1946).

WHEELER, C. E., CAWLEY, E. P., GRAY, H. T., CURTIS, A. C.: Gynecomastia: Review and analysis of 160 cases. Ann. intern. Med. **40**, 985 (1954).

WILLIAMS, M. J. W.: Gynecomastia. Amer. J. Med. **34**, 103 (1963).

WINN, E. B. LE: Gynecomastia during digitalis therapy. New Engl. J. Med. **248**, 316 (1953).

Testicular Hypertrophy and Testicular Tumors

Morbid Anatomy

ALBERTINI, A. v.: Histologische Geschwulstdiagnostik. Stuttgart: Georg Thieme 1955.

— Die Histogenese der Seminome. Schweiz. med. Wschr. **73**, 1091 (1943).

BRUNNER, K. W.: Prognose, Verlauf und Therapie der metastasierenden Hodentumoren. Schweiz. med. Wschr. **100**, 1359, 1376 (1970).

COLLINS, D. H., PUGH, R. C. B.: The pathology of testicular tumors. Brit. J. Urol. **36**, Suppl. to no. 2 (1964).

— SYMINGTON, T.: Sertoli-cell tumour. Brit. J. Urol. **36**, Suppl. to no. 2, 52 (1964).

CORNU, P.: Le pronostic des tumeurs testiculaires. Revue anatomo-clinique de 100 cas de classés d'après Collins et Pugh. Schweiz. med. Wschr. **101**, 422 (1971).

DIXON, F. J., MOORE, R. A.: Tumors of the male sex organs. Atlas of tumor pathology. Section VIII. Fascicles 31 b and 32. Washington, D. C.: A. F. I. P. 1952.

— — Testicular tumors. Cancer (Philad.) **6**, 427 (1953).

FEDERMAN, D. D.: Abnormal sexual development. Philadelphia and London: W. B. Saunders 1967.

HAMBURGER, CHR.: On the nature of gonadotrophin in cases of malignant tumors of the testis. Acta path. microbiol. scand. **18**, 457 (1941).

— GODTFREDSEN, E.: Studies on the excretion of androgenic substances and gonadotrophin in cases of malignant tumors of the testis, especially seminoma. Acta path. microbiol. scand. **18**, 485 (1941).

HEDINGER, CHR.: Beidseitige Hodentumoren und kongenitales adrenogenitales Syndrom (Leydig-Zellen oder Nebennierenrindengewebe?). Schweiz. Z. Path. **17**, 743 (1954).

— HUBER, R., WEBER, E.: Frequency of so-called hypoplastic or dysgenetic zones in scrotal and otherwise normal human testes. Virchows Arch. path. Anat. **342**, 165 (1967).

KARLY, M.: Orchioblastome ou adénocarcinome à cellules claires du testicule chez l'enfant. Helv. paediat. Acta **23**, 403 (1968).

LARON, Z., ZILKA, E.: Compensatory hypertrophy of testicle in unilateral cryptorchidism. J. clin. Endocr. **29**, 1409 (1969).

MORRIS, J. M., SCULLY, R. E.: Endocrine pathology of the ovary. St. Louis: C. V. Mosby 1958.

SALLE, B., HEDINGER, CHR.: Gonadal histology in children with male pseudohermaphroditism and mixed gonadal dysgenesis. Acta endocr. (Kbh.) **64**, 211 (1970).

SCHRÖDER, R., HEDINGER, CHR.: Paratestikuläre Tumoren. Schweiz. med. Wschr. **100**, 1281 (1970).

SCULLY, R. E.: Gonadoblastoma. A gonadal tumor related to the dysgerminoma (semioma) and capable of sex-hormone production. Cancer (Philad.) **6**, 455 (1953).

SIEBENMANN, R. E.: Pseudohermaphroditismus masculinus mit Gonadoblastom — eine besondere Intersexform. Path. Microbiol. (Basel) **24**, 233 (1961).

SYMINGTON, T., WALLACE, N.: Hormone investigations in cases of testicular tumour. Brit. J. Urol. **36**, Suppl. to no. 2, 103 (1964).

TEILUM, G.: Arrhenoblastoma — androblastoma. Homologous ovarian and testicular tumors. II. Acta path. microbiol. scand. **23**, 252 (1946).

— Estrogen-producing Sertoli-cell tumors (androblastoma tubulare lipoides) of the human testis and ovary. Homologous ovarian and testicular tumors. III. J. clin. Endocr. **9**, 301 (1949).
— Classification of testicular and ovarian androblastoma and Sertoli-cell tumors. Cancer (Philad.) **11**, 769 (1958).
— Special Tumors of ovary and testis and related extragonadal lesions. Comparative pathology and histological identification. Copenhagen: Munksgaard 1971.
WENZ, W.: Gutartiger Hodentumor mit einseitiger Gynäkomastie und Hypertonie. Schweiz. med. Wschr. **83**, 677 (1953).

Clinical Aspects

GOLDFINE, I., ROSENFIELD, R.L., LANDAU, L.: Hyperleydigism: A cause of severe puberal gynecomastia. J. clin. Endocr. **32**, 751 (1971).
GRANT, J.K., GRIFFITHS, K., PIERREPOINT, C.G.: Steroid biosynthesis in abnormal testes. Ciba Coll. Endocr. **16**, 280 (1967).
LIPSETT, M.J.: Metabolism of testosterone in metastatic interstitial cell carcinoma of the testis. J. clin. Invest. **45**, 1700 (1966).
RYSSEL, H.J., SONNTAG, R.W., BRUNNER, K.W.: Die internmedizinische Behandlung der Choriokarzinome und malignen Hodentumoren. Helv. med. Acta., Suppl. **47**, 112 (1967).
WEGIENKA, L.C., KOLB, F.O.: Hormonal studies of a benign interstitial cell tumor of the testis producing androstenedione and testosterone. Acta endocr. (Kbh.) **56**, 481 (1967).

Ectopic Formation of Gonadotropins

BOWER, B.S., GORDAN, G.S.: Hormonal effects of nonendocrine tumors. Ann. Rev. Med. **16**, 83 (1965).
FAIMAN, CH., COLWELL, J.A., RYAN, R.J., HERSHMAN, J.H., SHIELDS, T.W.: Gonadotropin secretion from a bronchogenic carcinoma. New Engl. J. Med. **277**, 1395 (1967).
FUSCO, F.D., ROSEN, S.W.: Gonadotropin-producing anaplastic large cell carcinomas of the lung. New Engl. J. Med. **275**, 507 (1966).
HUGUENIN, R., FAUVET, J., PIÉRART, A., LÉOBARDY, E.J. DE: Le syndrome "cancer bronchopulmonaire, périodite des os longs, gynécomastie". Bull. Soc. Méd. Paris **70**, 986 (1954).
STEINER, H., DAHLBAECK, O., WALDENSTROEM, J.: Ectopic growth hormone production and osteoarthropathy in carcinoma of the bronchus. Lancet **19681**, 783.

Methods of Investigation

Testosterone and evaluation of the hypothalamus-pituitary-gonadal axis.

BESSER, G.M., MACNEILLY, A.S., ANDERSON, D.C., MARSHALL, J.C., HARSOULIS, P., HALL, R., ORMSTON, B.J., ALEXANDER, L., COLLINS, W.P.: Hormonal responses to synthetic luteinizing hormone and follicle stimulating hormone-releasing hormone in man. Brit. med. J. **1972, 3**, 267.
CURTIUS, H.-CH., ZACHMANN, M., MÜLLER, M.: Gaschromatographische Bestimmung von Testosteron im Urin. Z. klin. Chem. **8**, 221 (1970).
FISCH, H.U., OLISKA, V., TESSER, G.L., SCHWYZER, R., ZACHMANN, M.: Simple assay of plasma testosterone using a covalent proteinsepharose complex. FEBS letters **29**, 127 (1973).
FOREST, M.G., CATHIARD, A.M., VERTRAND, J.A.: Total and unbound testosterone levels in the newborn and in normal and hypogonadal children: Use of a sensitive radioimmunoassay for testosterone. J. clin. Endocr. **36**, 1132 (1973).
KASTIN, A.J., GUAL, C., SCHALLY, A.V.: Clinical experience with hypothalamic releasing hormones. 2. Luteinizing hormone-releasing hormone and other hypophysiotropic releasing hormones. Recent Progr. Hormone Res. **28**, 201 (1972).
— SCHALLY, A.V., GUAL, C., ARIMURA, A.: Release of LH and FSH after administration of synthetic LH-releasing hormone. J. clin. Endocr. **34**, 753 (1972).
REBAR, R., YEN, S.S., VANDENBERG, G., NAFTOLIN, F., EHARA, Y., ENGBLOM, S., RYAN, K.J., AMOSS, M., GUILLEMIN, R.: Gonadotropin responses to synthetic LRF-dose-response relationship in men. J. clin. Endocr. **36**, 10 (1973).
RIVAROLA, M.A., BERGADA, C., CULLEN, M.: HCG stimulation test in prepubertal boys with cryptorchidism in bilateral anorchia and in male pseudohermaphroditism. J. clin. Endocr. **31**, 526 (1970).
ROTH, J.C., KELCH, R.P., KAPLAN, S.L., GRUMBACH, M.M.: FSH and LH response to luteinizing hormone-releasing factor in prepubertal and pubertal children, adult males and patients with hypogonadotropic and hypertropic hypogonadism. J. clin. Endocr. **35**, 925 (1972).
TAMM, J., LINDENMEYER, D.: The effect of intravenously infused synthetic LRH on LH, testosterone and dihydrotestosterone in the plasma of normal male subjects. Acta endocr. (Kbh.), Suppl. **173**, 91 (1973).
ZACHMANN, M.: The evaluation of testicular endocrine function before and in puberty. Effect of a single dose of human chorionic gonadotropin on urinary steroid excretion under normal and pathological conditions. Acta endocr. (Kbh.) **70**, Suppl. 164 (1972).

Testicular Biopsy

CHARNY, C.W.: Testicular biopsy. Its value in male sterility. J. Amer. med. Ass. **115**, 1429 (1940).
— MERANZE, D.R.: Testicular biopsy. Surg. Gynec. Obstet. **74**, 836 (1942).
CLAVADETSCHER, P., HEDINGER, CHR.: Wert von Hodenbiopsien bei Fertilitätsstörungen und adultem Hypogonadismus. Schweiz. med. Wschr. **100**, 732 (1970).
ENGLE, E.T.: Testis biopsy in infertility. J. Urol. (Baltimore) **57**, 789 (1947).
HEDINGER, CHR.: Diagnostischer und prognostischer Wert der Hodenbiopsie. Schweiz. med. Wschr. **101**, 1084 (1971).
HOTCHKISS, R.S.: Etiology and diagnosis in the treatment of infertility in men. Springfield: Ch.C. Thomas 1952.
JORDAN, D.: Hodenhistologie bei Oligospermie in Abhängigkeit zur Spermatozoenzahl. Stuttgart: Enke 1966.
KAPLAN, E., SHWACHMAN, H., PERLMUTTER, A.D., RULE, A., KHAW, K.-T., HOLSCLAW, D.S.: Reproductive failure in males with cystic fibrosis. New Engl. J. Med. **279**, 65 (1968).
NELSON, W.O.: Testicular morphology in eunuchoidal and infertile men. Fertil. Steril. **1**, 477 (1950).
— Male infertility. In: GOLDZIEHER, Endocrine treatment in general practice. New York: Springer 1953.
— HELLER, C.G.: The testes in human hypogonadism. Recent Progr. Hormone Res. **3**, 197 (1948).
PAYNE, S., SHEELS, R.F.: Testicular biopsy: which infertile patients are benefited. Fertil. Steril. **6**, 43 (1955).
ROULET, F.: Methoden der pathologischen Histologie. Wien: Springer 1948.
SCHUCHARDT, E.: Zur qualitativen Beurteilung menschlicher Hodenbiopsien. In: NOWAKOWSKI, Die Keimdrüsen des Mannes. Berlin-Göttingen-Heidelberg: Springer 1955.
SNIFFEN, R.C.: The testis. I. The normal testis. Arch. Path. **50**, 259 (1950).

SNIFFEN, R.C., HOWARD, R.P., SIMMONS, F.A.: The testis. II. The abnormalities of spermatogenesis, atresia of the excretory ducts. Arch. Path. **50**, 258 (1950).
— — — The testis. III. Absence of germ cells, sclerosing tubular degeneration, "male climacteric". Arch. Path. **51**, 293 (1951).
TONUTTI, E., WELLER, O., SCHUCHARDT, E., HEINKE, E.: Die männliche Keimdrüse, Stuttgart: Thieme 1960.
VALMAN, H.B., FRANCE, N.E.: The vas deferens in cystic fibrosis. Lancet **1969 II**, 567.
WEYENETH, R.: La biopsie du testicule, son indication et sa valeur clinique. Gynaecologia (Basel), Suppl. **134** (1952).
— Über histologische Befunde (Hodenbiopsie) bei Aspermien-Azoospermien und deren Behandlung. Arch. Gynäk. **184**, 420 (1954).

References about methods of investigation semen see under sterility.

For the methods of determination of testosterone see also Chap. VII, p. 384, 390, for those of estrogens and gonadotropins Chap. X, p. 547 and p. 562. Methods for mesurements of body constitution, development and testicular size in Chap. XIX.

X. The Ovary

W. E. SCHREINER

A. Historical Dates

1668 N. STENSEN described the ovary as the organ in which ova are formed.

1672 R. DE GRAAF and TH. KERCKRING observed the presence of the graafian follicle and yellow body in the ovary of the woman.

1686 M. MALPIGHI coined the term "corpus luteum" and suggested that this structure had characteristics of a gland.

1778 A. VON HALLER described the conversion of the follicle into the corpus luteum.

1827 K. E. VON BAER discovered the human ovum.

1871 R. SIGISMUND, a general practitioner, showed that there was mucosal destruction during menstruation, and deduced that menstruation resulted when fertilization failed to occur.

1873 H. KUNDRAT and G. J. ENGELMANN discovered the cyclic changes in the endometrium.

1893 E. RÉGIS used ovarian extracts injected subcutaneously to demonstrate that the ovary produced internal secretions.

1896 E. KNAUER and HALBAN (1900) showed the endocrine action of the ovary on the genital organs in animal experiments by implanting ovaries in castrated rabbits.

1898 L. A. PRENANT and BORN (1900) suspected a connection between the corpus luteum and pregnancy, since the corpus luteum inhibits ovulation.

1902 L. FRAENKEL showed that excision of the corpus luteum in the early stages of pregnancy led to abortion in rabbits.

1905 F. H. A. MARSHALL and W. A. JOLLY discovered that ovarian extracts produced estrus in castrated animals.

1911 L. FRAENKEL observed that there was a time relation between ovulation and menstruation.

1910–1913 L. ADLER (1910) and O. FELLNER (1912, 1913) succeeded in obtaining extracts from the ovary and placenta, which caused estrus in castrated animals.

1913–1915 L. FELLNER (1913) and E. HERMANN (1915) produced extracts from the corpus luteum and placenta.

1913–1915 R. MEYER and R. SCHRÖDER recognized the morphological connection between ovarian function and endometrium.

1917 C. R. STOCKARD and G. N. PAPANICOLAOU described the cyclic changes in the vagina of guinea pig.

1921 H. M. EVANS and J. A. LONG found the first indication of a gonadotropic principle of the anterior pituitary lobe in rats.

1923 E. ALLAN and E. A. DOISY recognized that signs of estrus were dependent on ovarian function in mice and rats. They obtained a relatively purified crystalline substance from bovine and porcine follicular fluid, and were able to induce estrus in rats with this substance. They elaborated this observation to produce a test, which was named after them, and thus derived the first exact hormone estimation.

1926–1927 R. T. FRANK demonstrated the estrus-inducing hormone in the blood by means of the Allen and Doisy test. S. ASCHHEIM and B. ZONDEK showed the presence of the same substance in the urine of pregnant animals.

S. ASCHHEIM, B. ZONDEK, and P. E. SMITH obtained proof from animal experiments with transplantation after hypophysectomy that ovarian function was dependent on the anterior pituitary lobe. The hypothesis of two different gonadotropins was then developed. B. ZONDEK and S. ASCHHEIM (1928) demonstrated successfully that there was a follicular-stimulating principle in the urine of menopausal women.

1929 E. A. DOISY and A. BUTENANDT reported almost at the same time on the isolation of an estrogen-active substance in crystalline form the urine of pregnant women. N. K. ADAM suggested that this substance be named estrone because of the C-17-ketone group present (1933).

Chemical research on the estrogen hormones began at this time. G. W. CORNER and W. M. ALLEN noticed that termination of pregnancy at an early stage in rabbits due to destruction of the corpus luteum failed to occur if extracts of corpus luteum were injected (porcine).

1929/30 H. KNAUS (Graz/Prag) reported that follicular maturation was inhibited by extracts of corpus luteum, and that sensitivity of uterine muscle to extracts of the posterior pituitary was also reduced.

1930 A. BUTENANDT *et al.* determined the structural formula of estrone. G. F. MARRIAN and E. A. DOISY extracted estradiol in crystalline form from the urine of pregnant women.

C. CLAUBERG showed that progesterone produced no proliferative action on the endometrium but an entirely differentiating effect, and that it is only effective on endometrium undergoing proliferation due to estrogens.

E. PHILIPP showed experimentally that progesterone was produced in large amounts in the syncytium of the chorion during pregnancy. G. T. POPA and U. FIELDING described the portal vascular system connecting hypothalamus and hypophysis.

1931 P. E. CLAUS and H. E. FEVOLD and their teams produced potent extracts from anterior pituitaries.

1932 A. BUTENANDT showed that esters of estrone had prolonged activity. Treatment with estrogens commenced at this stage.

C. KAUFMANN referred knowledge gained from experiments in rabbits to the castrated woman.

W. HOHLWEG and K. JUNKMANN postulated that there was a higher "sexual center" in the hypothalamus.

1934 S. L. COHEN and G. F. MARRIAN described a chemical method based on KOBER's method for estimation of estrogens in the urine.

W. SLOTTA, W. RUSCHNIG, and W. FELS succeeded in purifying the hormone of the corpus luteum. A. BUTENANDT and U. WESTPHAL successfully determined the structure of this hormone. It was officially named *progesterone* because of its protective action during pregnancy.

1935 MCCORQUODALE and his team isolated estradiol from the ovaries of pigs.

1938 H. H. INHOFFEN and W. HOHLWEG produced the first highly potent oral estrogen (ethinyl estradiol) from estrone. At the same time they succeeded in synthesizing an orally effective gestagen (ethinyl testosterone = pregneninolone).

1940 H. H. INHOFFEN succeeded in partially synthesizing estradiol from cholesterol. Pure progesterone was produced from cholesterol.

1946 J. E. MARKEE and his team demonstrated experimentally the humoral control of the hypophysis through hypothalamic "action substances" (so-called neurovascular chain).

1954 C. DJERASSI and his team synthesized 19-norsteroids which were then introduced therapeutically as orally effective progestines by R. HERTZ and his team.

1958 J. ZANDER discovered another natural gestagen, 20α,β-hydroxypregn-4-ene-3-one, in the placenta, corpus luteum and mature follicle.

From 1955 G. F. MARRIAN and his team described a series of other natural estrogens representing metabolites.

1955 G. W. HARRIS and his team and R. GUILLEMIN, S. M. MCCANN, M. SAFFRAN, and A. V. SCHALLY showed in animal experiments that hypothalamic extracts caused release of gonadotropins from the anterior pituitary.

1971 A. V. SCHALLY and his team isolated LRH and determined its chemical structure.

B. Anatomy

1. Macroscopic Anatomy

There are changes in position, size, form, color and superficial structure of the ovaries during the different phases of life. In the newborn, they are found on the posterior surface of the uterus above the linea innominata. During childhood they migrate into the small pelvis and come to lie on the lateral wall of the small pelvis at the beginning of puberty. They become almond-shaped at the stage at which increase in height occurs. The ovary of the newborn is pink and shiny and the surface is smooth. At puberty it is grey in color and shows cystic protrusions corresponding to tertiary follicles which measure 3–8 mm in diameter. The ovary reaches its full size in the 25th year. It is then 2.5–5 cm long, 1.5–3 cm wide, 0.6–1.5 cm thick and weighs 7.2–14.6 g (WATZKA, 1957).

During pregnancy the ovaries are displaced from the small pelvis by the enlarging uterus and have a reddish felt-like covering at the end

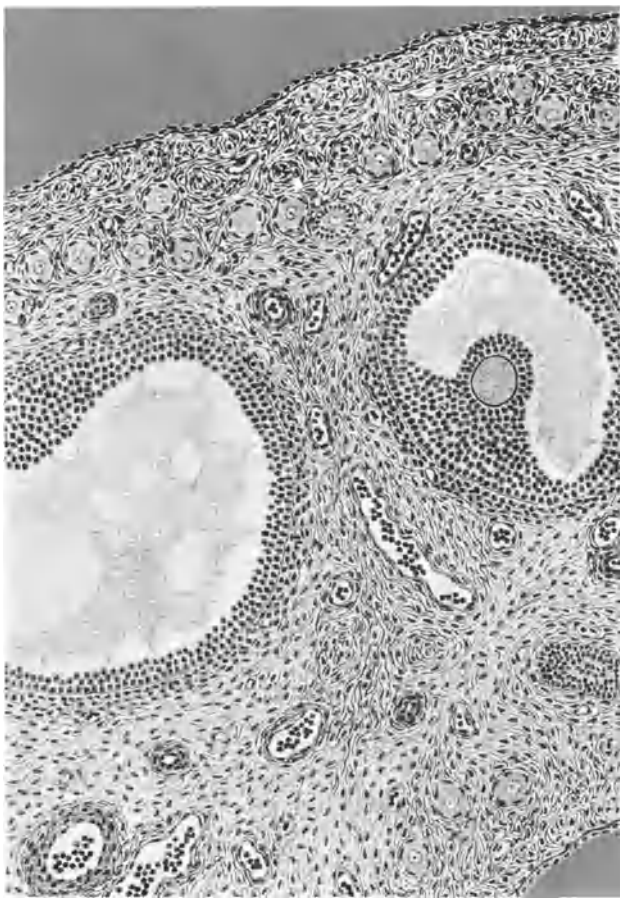


Fig. 1. Section of an ovary from a prepubertal girl. Numerous primordial follicles can be seen in the corticalis (zona parenchymatosa), as can a primary follicle in the process of developing into a secondary follicle and two tertiary follicles at different stages of development, neither of which has attained full maturity

of pregnancy. This covering is due to the reaction of the "germinal epithelium" to stimulation by estrogens and gestagens. The ovary shrinks to about one third its original size during the post menopause, when it is white in color and has numerous scarred depressions on its surface.

There are two ovaries. The flattened ventral border of each ovary is bound by the mesovarium to the posterior surface of the ligamentum latum. The mesovarium ends on the ovarian surface at the level of the border between the hilus and medulla, at the so-called *Farré-Waldeyer's line*, which forms the boundary between the ovarian surface covered with peritoneum and that free of peritoneum. The free surface is covered by the so-called germinal epithelium, consisting of one layer of cuboid to cylindrical epithelial cells. Peritoneum and germinal epithelium are of the same origin. They both arise from the mesodermal coat of the embryonal coelom (mesothelium). It appear, however, that the prospective potency of the so-called germinal epithelium remains considerably higher than that of the rest of the peritoneum. It is currently assumed that the majority of ovarian tumors, such as serous and pseudo-mucinous cystomas and endometrioid tumors, arise from the germinal epithelium.

In the adult the ovary is kept in the vertical position by the mesovarium, the fibromuscular utero-ovarian cord and the infundibulopelvic ligament.

As a rule, the right ovary, which is phylogenetically older, is somewhat larger than the left.

Supernumerary ovaries are extremely uncommon (6 cases in the world literature) (VALDES-DAPENA, 1967). They may lie in the ligamentum latum, in the infundibulopelvic ligament or retroperitoneally. There is usually an associated uterine malformation.

These ovaries must be differentiated from *accessory ovaries*, which are more common and usually lie in the immediate vicinity of an ovary, and occasionally even connected with it. Uterine malformation is exceptional in these cases.

Absence of one ovary is almost without exception associated with developmental disturbances of Müller's tube giving rise to homolateral aplasia of the tube and uterine cornus. Bilateral aplasia, so-called *gonadal agenesis* is a rarity (ERSKINE, 1946; OVERZIER, 1961). Rudimentary gonads consisting of stroma and with no sex cells are found considerably more frequently (gonadal dysgenesis). Clinically, a differentiation is made between different syndromes with gonadal dysgenesis, depending on the associated growth disorder and malformations:

Gonadal dysgenesis + normal growth
= pure gonadal dysgenesis or Swyer's syndrome

Gonadal dysgenesis + small stature
= Rössle's syndrome

Gonadal dysgenesis + small stature + malformation
= Turner's syndrome.

Gonadal dysgenesis must also be differentiated from *ovarian hypoplasia*, where the small ovaries are also often cyclindrical in shape. A few primordial follicles can, however, be found in normal ovarian stroma (STAEMMLER, 1964) (p. 46).

In true hermaphroditism, ovaries and testes are present together, either separately or as ovotestes.

Exceptionally, the ovary fails to descend on one side or on both sides. The ovary may then lie under the liver. The ovary occasionally lies in the inguinal canal or in the labium majus (congenital hernia). It cannot be placed back since the mesosalpinx changes immediately into the hernial sack.

The density of the fibrous tissue structures increases from the inside out. The *medulla* of the ovary consists of loose, vascular and nervous structures (zona vasculosa). *The cortex* is denser and contains many cells (zona parenchymatosa) and the *tunica albuginea* is tough (Figs. 1–4). The medulla contains the rete

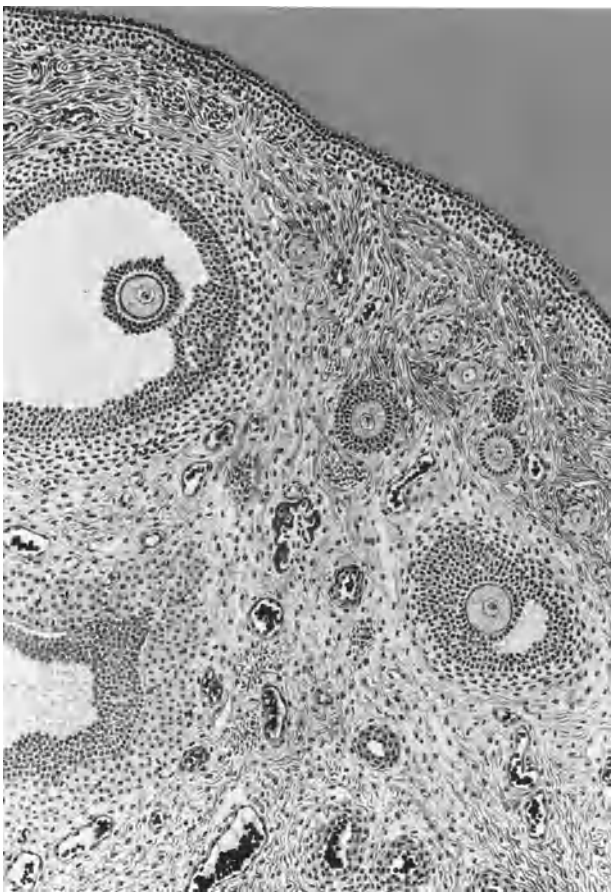


Fig. 2. Section of an ovary from a woman of 19 or 20. Primordial, primary and secondary follicles and a graafian follicle are seen in the corticalis, and a tertiary follicle is beginning to form. A corpus luteum and a corpus atreticum are also present

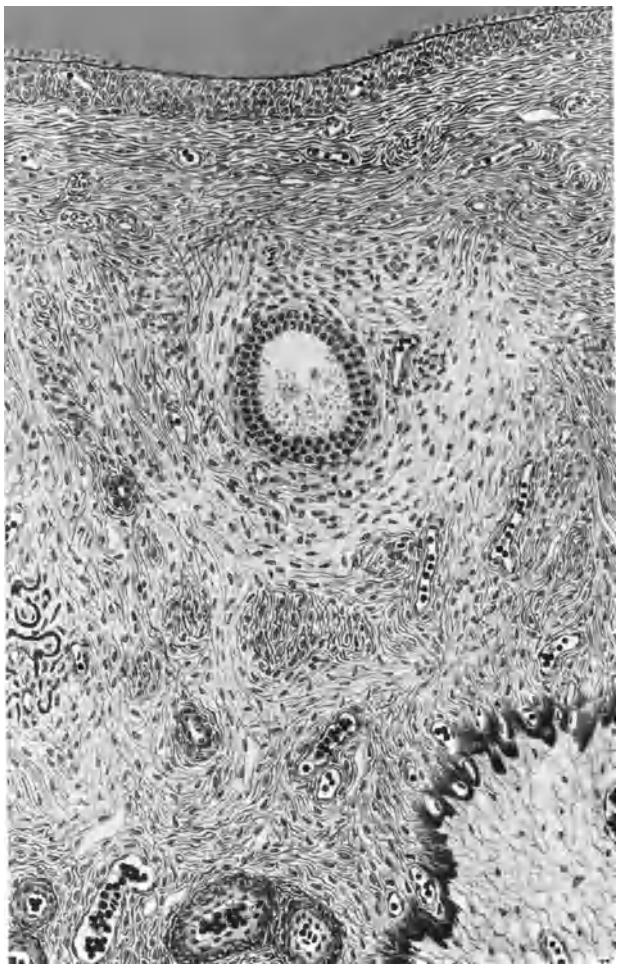


Fig. 3. Section of an ovary from a premenopausal woman. The corticalis contains only isolated primordial follicles or none at all; one tertiary follicle is seen to be disintegrating (pyknosis, disintegration of the ovum, dissociation). One corpus luteum is at the stage of conversion to a corpus albicans

ovarii, consisting of a few degenerated and functionless tubules, whereas the cortex contains the ova which form the germinal parenchyma. The tunica albuginea possesses a dense frame of collagen fibers running parallel to the ovarian surface and fixed with ramifications from the surface to the underlying mesenchyme. On the other side, delicate argyrophilic fibers surrounding the individual follicles radiate into the tunica albuginea.

Blood, lymph vessels, and nerves leave and enter the ovary at the ovarian hilus situated on the ventral border of the ovary. It contains about 80% of all the large ovarian polyhedral eosinophilic cells. These cells store lipochrome, and correspond morphologically and probably also functionally to the Leydig cells of the testis. These cells are arranged in groups and are closely connected topographically to unmedullated nerve fibers, blood and lymph vessels. They are described as *hilus cells*. Since hyperplasia of these cells and tumors arising from these cells are associated with virilization, it has been assumed that they produce androgens (MERRILL, 1959). They are found in increased amounts in anencephalic fetuses. It is possible that this hilus-cell hyperplasia is a compensytory process due to the adrenal atrophy present with anencephaly.

The changing vascular demand of the functioning ovary is insured by balanced vascularization, which self-regulates blood pressure and blood distribution. Thus for example, the anti-clockwise arterial vessels are stretched during follicular maturation in this region. This causes vascular resistence to be reduced and circulation increased. Local blood supply is also regulated by arteriovenous anastomoses and barrier arteries (WATZKA, 1957). The great abundance of lymph vessels is probably connected with the formation of follicular fluid.

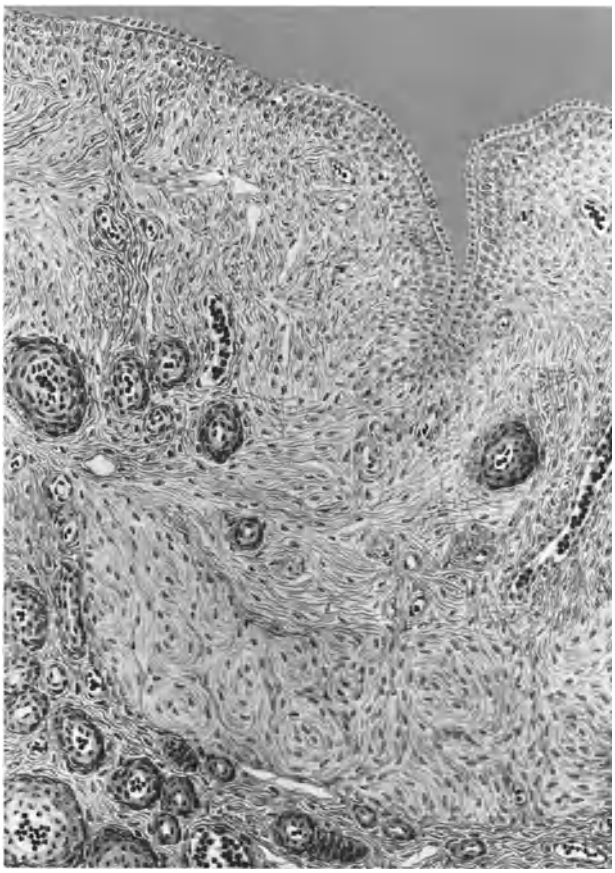


Fig. 4. Section of an ovary from an elderly woman. Follicles are no longer present in the corticalis, and only corpora albicantia in the medulla (zona vasculosa). Intense proliferation of the intima has caused thickening of the walls of small and medium-sized arteries and hyalin degeneration of some vessel walls

2. Embryology and Histology

The germinal cells can be recognized in the region of the allantois in the 4-week human embryo. From this region, several hundred of these cells migrate by means of ameboid movements (so-called entodermal migrating cells) and are chemotactically conducted into the germinal ridge, which strictly speaking should be termed the genital ridge. The genital ridge develops as a thickening of coelomic epithelium near the mesonephros and the adrenocortical "anlage". Ovarian mesenchyme is thus mesodermal in origin.

Between the 7th and 8th weeks of pregnancy, the fetal gonads begin to undergo sexual differentiation. The ovary can be structurally distinguished from the testis at this stage, since it has quite a wide cortex separated from a small medulla. At the same time, the oogonia which have migrated via the germinal ridge begin to divide mitotically. The original few hundred cells develop into 5–6 million cells within 2–3 weeks.

The phases of multiplication and growth of the oogonia (STIEVE, 1943, 1952) appear to be concluded in the human in the 10th week of pregnancy (1st phase of growth). Nuclear maturation then begins, and oogonia are converted into oocytes (10th to 20th week of pregnancy). Oogonia can no longer increase in number after the 20th week of pregnancy. Postnatal oogenesis has been widely discussed, but it has never been proved to occur and it is therefore rejected by most authors (STIEVE, 1952; WATZKA, 1957; ZUCKERMANN, 1951, 1957). The *primary oocyte* developed from the oogonium is in the prophase of the first *reduction division* or *meiosis* at birth. It remains at this stage for many years, at least until sexual maturity (1st resting phase). The first meiosis is resumed during postnatal development of the follicle and is ended about the time ovulation occurs. This may take as long as 12–50 years after the onset of meiosis. Reduction division gives rise to the *secondary oocyte* and polar body. Each possesses 23 chromosomes. The *2nd meiosis* occurs immediately after the first

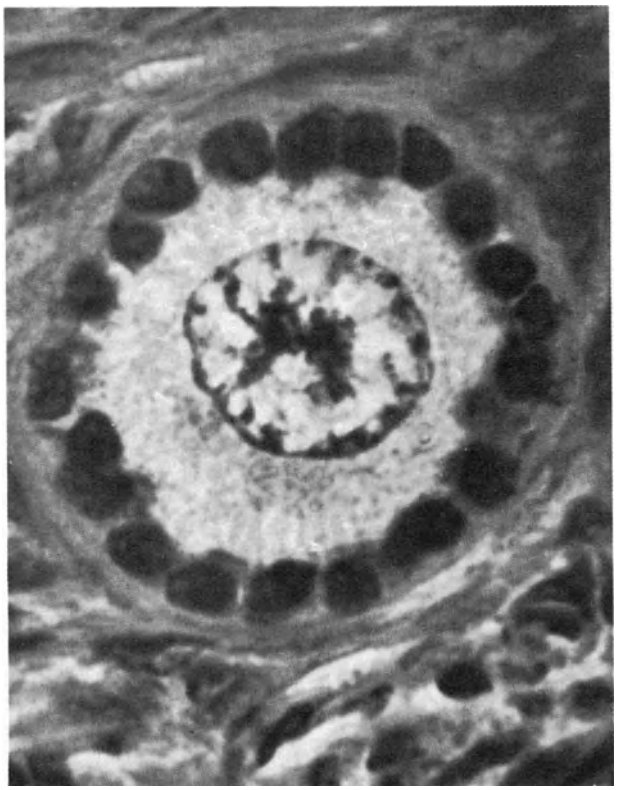


Fig. 5. Primordial follicle (human). (After SHETTLES, 1960)

and ends only after fertilization. It gives rise to the mature ovum and one polar body. From about the 20th week of pregnancy, the primary oocyte is surrounded by a single layer of cuboid cells, follicular or granulosa cells. It is currently assumed that these cells arise from the germinal epithelium. Oocyte and follicular cells together form the so-called primordial follicle (Fig. 5).

The two ovaries of the newborn contain about 500000–700000 primordial follicles which are closely packed together in the zona parenchymatosa. The majority undergo destruction due to atresia even during the intra-uterine phase. At menarche, there are still about 400000 primordial follicles, while at age 35, there are only about 80000 (Table 1). Only 300–400 primordial follicles develop into mature follicles with the potential possibility of becoming fertilized during sexual maturity. About 90% of the primordial follicles become atretic at all stages of development from the postnatal stage right up to the onset of the postmenopause. Both ovaries together contain less than 10000 primordial follicles at the onset of postmenopause. If these follicles are transplanted into ovaries of young sexually mature animals, they can grow into mature follicles.

Table 1. Quantities of follicles in the human ovaries as a function of age (BLOCK, 1952)

Age (years)	No. of cases examined	Average no. of primordial follicles in both ovaries	Average no. of growing follicles (up to and including 100 μm) in both ovaries	Average no. of graafian follicles in both ovaries
6– 9	5	484000 (258000–755000)	15400 (7700–34600)	13– 97
12–16	5	382000 (85000–591000)	7300 (3000–11200)	231
18–24	7	155000 (39000–290000)	6800 (1000–17300)	27–136
25–31	11	59000 (8100–228000)	3500 (680– 9200)	21–117
32–38	8	74000 (15000–208000)	6200 (1800–16500)	33–101
40–44	7	8300 (350– 28000)	2600 (900– 9400)	11– 81

In parentheses: minimum and maximum numbers.

Thus, the follicles do not grow old, but the stroma probably does, possibly due to vascular changes (p. 580). Generative ovarian function disappears with failure of the follicle to react to increased gonadotropic stimulation.

Ovarian Cycle. The primordial follicle has a diameter of 40–60 mμ and contains a primary oocyte measuring 18–24 mμ in diameter, which is surrounded by a layer of cuboid follicular cells (Fig. 5). Even during the fetal phase, childhood, and especially at the time of puberty, single primordial follicles lying deep in the cortical layers grow quickly into primary, secondary, and tertiary follicles (2nd phase of growth). They protrude into the superficial layers, and have a diameter of up to 3 mm (Fig. 2). They develop before the 5th year, probably without the influence of gonadotropins (cf. hypophyseal gonadotropins p. 559). They can remain at this stage for several months (2nd resting phase).

We do not know which factors induce the resting primordial follicle to grow, nor do we know whether the primary stimulus occurs in the oocyte or in the follicular cells. It appears that the second phase of growth is partly independent of the pituitary since a certain degree of follicular growth also occurs in hypophysectomized animals.

The cuboid follicular cells grow into cylindrical cells, and the oocyte enlarges at the same time to produce the *primary follicle*. The follicular cells increase in number and size to form the *secondary follicle*. A membrana granulosa of 3–4 layers, containing numerous mitoses, is formed. The secondary follicle has a maximum diameter of 0.3 mm. During this period, the ovum develops into the largest cell of the organism (90–130 μm diameter). After this stage is reached, only the follicle continues to grow.

Until the onset of sexual maturity, any tertiary follicles formed become atretic. Atresia begins in the granulosa cells. The tertiary follicle is characterized by the formation of an antrum, which develops through confluence of small single vesicles, and is filled with follicular liquor. This is probably due to active secretion of the granulosa cells. Labeled water appears first in the granulosa cells and then in the follicular fluid. It is possible that cellular degeneration and vascular transudation from the follicular veins, which become congested through growth of the follicle, also has some effect. The follicular fluid has a high content

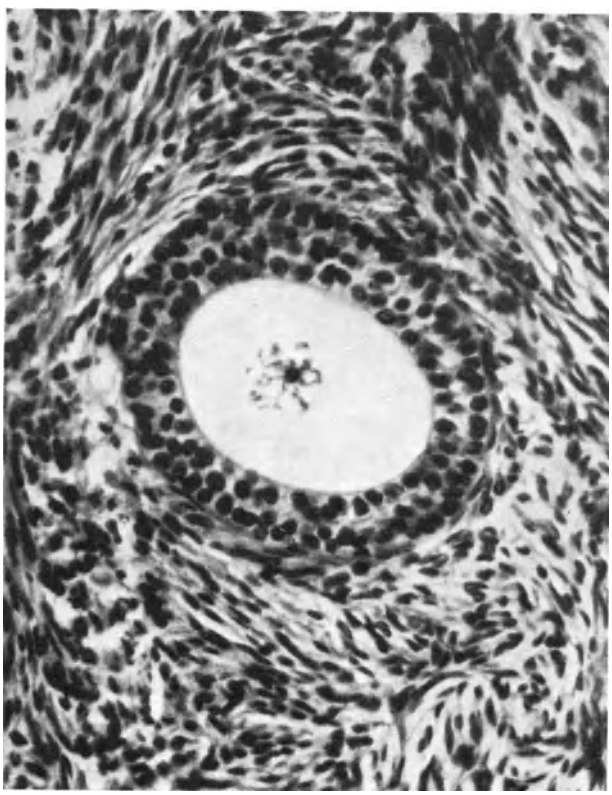


Fig. 6. Secondary follicle (human). (After SHETTLES, 1960)

of estrogens (estrone and estradiol), and also of progesterone from mid-cycle on. Microscopically small collections of fluid can be seen between the granulosa cells, and together with the neighboring follicular layer these form the so-called *Call-Exner body*. The cell margins of the granulosa cells become gradually indistinct, and 8–12 layers of these cells form the *membrana granulosa*. It is thickened at one point, where the ovum is contained *(cumulus oophorus, discus proligerus)*. The granulosa cells next to the oocyte form the *corona radiata*. Its cells are characterized by y chromatin-rich nucleus and granular cytoplasm. They become cylindrical and adopt a radial arrangement. They form a biological unit with the oocyte, and the hyaline *zona pellucida* of 5 mμ thickness surrounding the ovum is pierced by processes of the cells of the corona radiata. The intact membrana granulosa contains no vessels or nerves. Connective tissue structures appear for the first time in the tertiary follicle, in the form of the theca interna and externa. The theca interna is composed of delicate fibrils of connective tissue with a rich supply of capillaries and nonmedullated nerve fibers, and also contains spiral cells (fibrocytes) and clear, polygonal theca-lutein cells laden with fat. The latter cells are particularly well-developed in follicles reaching ovulation and in polycystic ovaries. They probably contain steroids which are precursors of estrogens and gestagens. They also show increased dehydrogenase activity (JKONEN, 1961). The theca-lutein cells arise from the mesenchyme, probably originating from the ovarian medulla.

The theca externa is composed of strong collagen fibers arranged concentrically, and contains blood and lymph vessels. It surrounds the tertiary follicle, which has a diameter of 3–8 mm at this stage. A fibrous membrane separates the vascular theca interna from the avascular membrane granulosa. During sexual maturity, single tertiary follicles grow from the 8th to 10th day of the cycle (CORNER, 1952) into *Graafian follicles* (Fig. 2), which have a diameter of 16–24 mm. Whereas there is a regular increase in the size of the follicle during the phase of follicular maturation, growth is greatly accelerated (Fig. 7) towards the ovarian surface (BLANDAU, 1966) 10–12 hours prior to ovulation (rats). The theca is pushed forward in the form of a wedge against the ovarian surface (thecal wedge), so that the follicle finally protrudes conically from the ovarian surface. Terminal growth is due mainly to an increase in follicular fluid, which becomes less viscous at the same time due to depolymerization (BLANDAU, 1966). The number of mitoses decreases in the membrana granulosa, which is still avascular and which at this stage has 16–20 layers of cells. The cells of the cumulus oopherus steadily dissociate, but the corona radiata remains intact. The gel of the ovum is released from the membrana granulosa. The theca interna becomes hyperemic and loose, and *K cells* arise which are eosinophilic and contain a dark nucleus. These cells are said to possess a special secretory function, but nothing definite is known about the nature of this function.

Nor are we fully orientated about the original physical and chemical factors involved in ovulation. It is very probable that follicular rupture is not only due to a rise in intrafollicular pressure, and that vascular and enzymatic processes are also very much involved (MORICARD, 1946; BLANDAU, 1966, 1967). Follicular fluid contains high concentrations of proteolytic enzymes. It is not yet known what effect gonadotropins exert in this process (JUNG, 1959). The *follicular stigma* is the thinnest spot of the Graafian follicle lying against the ovarian surface, and before ovulation consists of only 1–2 layers of granulosa and theca cells and diluted tunica albuginea. The stigma becomes anemic before ovulation and is pale yellow in color. Digestion by proteases together with a slight rise in intrafollicular pressure probably

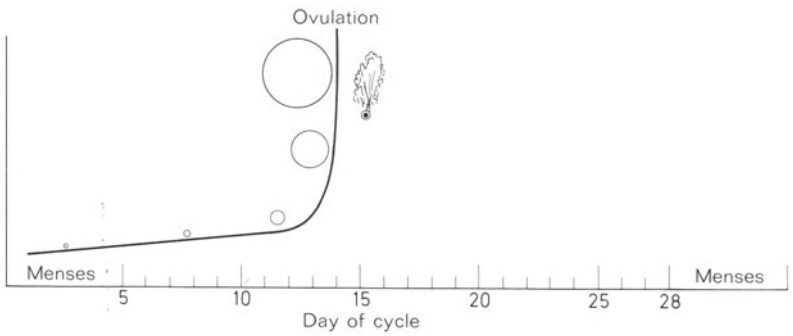


Fig. 7. Growth curve of the follicle, showing the spurt of growth beginning 10 hours before ovulation. (After HARTMAN, 1962)

then causes follicular rupture. Ovulation has been observed in rats and rabbits (WALTON, 1928; BLANDAU, 1955). This does not occur by explosive bursting of the mature follicle, but slowly through gradual release of a viscous fluid, which flows from the stigma of the follicle as from an hourglass. The ovum is finally flooded out together with the cumulus ovigerus. Fibrillary currents and tubal peristalsis convey the ovum to the tubal ampulla, where fertilization may occur. The second meiosis occurs during this sequence of events, but only comes to an end after fertilization. Bleeding connected with follicular rupture leads to closure of the site of rupture through formation of blood clots which also fill up the collapsed follicular lumen. The bleeding is usually very mild. It is questionable whether "mittelschmerz" is caused by slight peritoneal irritation due to this bleeding (ISRAEL, 1967).

The corpus luteum develops from the ruptured follicle (CORNER, 1956). According to MEYER'S (1911, 1913) classic description of the formation of the corpus luteum (which is still valid) 4 developmental stages can be differentiated. First, there is intensive growth of the granulosa, and of theca cells in particular. These cells contain large amounts of lipoids (proliferative stage). Vascularization of the membrana granulosa occurs after 3 to 4 days. Capillaries, connective tissue and K cells penetrate right into the center of the coagulum from the theca interna. Nearly every granulosa cell is thus directly connected with a vessel. Macroscopically, the dark red periphery is clearly distinguishable from the pink, gelatinous center. During the budding phase (from the 5th to about the 10th postovulatory day) the corpus luteum increases in size again; the granulosa cells grow and are now arranged in columns, forming a reddish yellow covering zone around the sanguine gelatinous center. The yellow color is due to carotin, and the various color tones are caused by the oxidation product of carotin. The granulosa cells are polygonal, have a clear bright cytoplasm and can be clearly differentiated from the smaller, even brighter cells of the theca interna interspersed among them. While connective tissue grows inwards, the central core steadily liquefies. This stage is reached after not more than 7 days. If fertilization does not occur the corpus luteum undergoes rapid regression 3–4 days before menstruation (about the 10th postovulatory day) due to the absence of the luteotropic action of chorionic gonadotropin. The center becomes fibrous, and then hyaline, as does the fibrous septa between the granulosa cells. The granulosa cells themselves become smaller, granular and vacuolized, and lose their columnar arrangement. The loosely arranged theca cells show a dark nucleus in pale cytoplasm. The theca interna becomes fibrotic and finally undergoes hyaline degeneration. The *corpus albicans* is formed by a process of shrinking within roughly 70 days. It contains cells with iron pigment, a residue of the central bleeding. A fibrous depression is produced through shrinking. This area is characterized by a broad hyaline zone which is sharply defined and undulated, and surrounds a fibrous nuclear zone (Fig. 3).

The majority of follicles regress long before reaching maturity *(follicular atresia)* (SLAVJANSKY). This regressive process even occurs in the ovary of the fetus during the second half of pregnancy, and is also particularly pronounced during pregnancy. An oocyte must be completely surrounded by follicular cells to prevent its becoming atretic. If this is not so, there is a hurried maturation process with subsequent atresia (OHNO, 1964; BLANDAU, 1965). Atresia probably starts in the oocyte, resulting in chromatolysis of the germinal vesicle with subsequent round-cell invasion of the degenerating ovum and dissociation of the cumulus oopherus. The liquor folliculi is absorbed, the peripheral cell layers fold, the basal membrane becomes thickened and hyaline, and the granulosa cells and some theca cells degenerate and are finally replaced by fibrous tissue. This gives rise to the *corpus fibrosum*. The scar formed in this way can persist for years as a corpus fibrosum or atreticum. This corpus have purely hyaline structure or can be an undulating hyaline "glass skin" surrounding a reticular fibrous tissue. Cells containing iron are also absent. Some of the cells of the theca interna of the atretic secondary and tertiary follicles remain intact and together form the so-called "interstitial gland" or the *theca gland* of the ovary (TONUTTI, 1961). This "gland" probably secretes small amounts of estrogens continuously (basal estrogen formation) (DEANE, 1952). The theca glands react to the luteinizing hormone, which is also termed the interstitial cell-stimulating hormone by increasing in size. It is thus not astonishing that the epitheliod theca lutein cells also increase in size during pregnancy (SCHRÖDER, 1930). This reaction is especially pronounced in polycystic ovaries and in the adrenogenital syndrome, and in the latter case is related to a rise in the production of androgens. Primary follicles disappear completely. During the post menopause the ovary shrinks to half or one third of its original size (WATZKA, 1957). The few follicles still present become atretic within 4–5 years

(LAURITZEN, 1968). During this time, follicular maturation and ovulation occasionally occur, with formation of a corpus luteum and uterine bleeding from a transformed endometrium.

C. Physiology of Ovarian Function

Ovarian Hormones (Natural and Synthetic)

1. Chemistry and Occurrence

Results obtained by isolating steroids from human ovaries (ZANDER, 1958, 1959; RYAN, 1967) and estimating their concentrations in ovarian veins and peripheral blood (MIKHAIL, 1967) have shown that the following steroid hormones are formed and secreted by the normal ovary of the woman (Fig. 8, Table 2): progesterone, 17α-hydroxyprogesterone, 20α,β-hydroxy-4-pregnen-3-one, dehydroepiandrosterone, androstenedione, testosterone, estrone, estradiol and estriol.

Chemically these hormones are steroids *(sexual steroids)*. The basic framework is the sterane or gonane, constisting of 3 hydrated benzol rings (A, B, C) and a cyclopentane ring (D). Structure and numbering of the C atoms is shown in Fig. 8 (cyclopentanoperhydrophenanthrene).

The biological action of the sexual steroids is very much dependent on their stereochemical structure. An exact description is therefore necessary (Chap. VII, p. 287). Three groups of ovarian steroids are differentiated according to their biological action (p. 532ff.): gestagens, androgens and estrogens (Fig. 8).

Table 2. Steroids isolated from normal human ovary (RYAN, 1967)

Follicular fluid	Corpera lutea
Estradiol	Progesterone
Estrone	20α-hydroxypregn-4-en-3-one
Estriol	17α-hydroxyprogesterone
Androstendione	Androstendione
17α-hydroxyprogesterone	Estradiol
20α-hydroxypregn-4-en-3-one	Estrone
Progesterone	

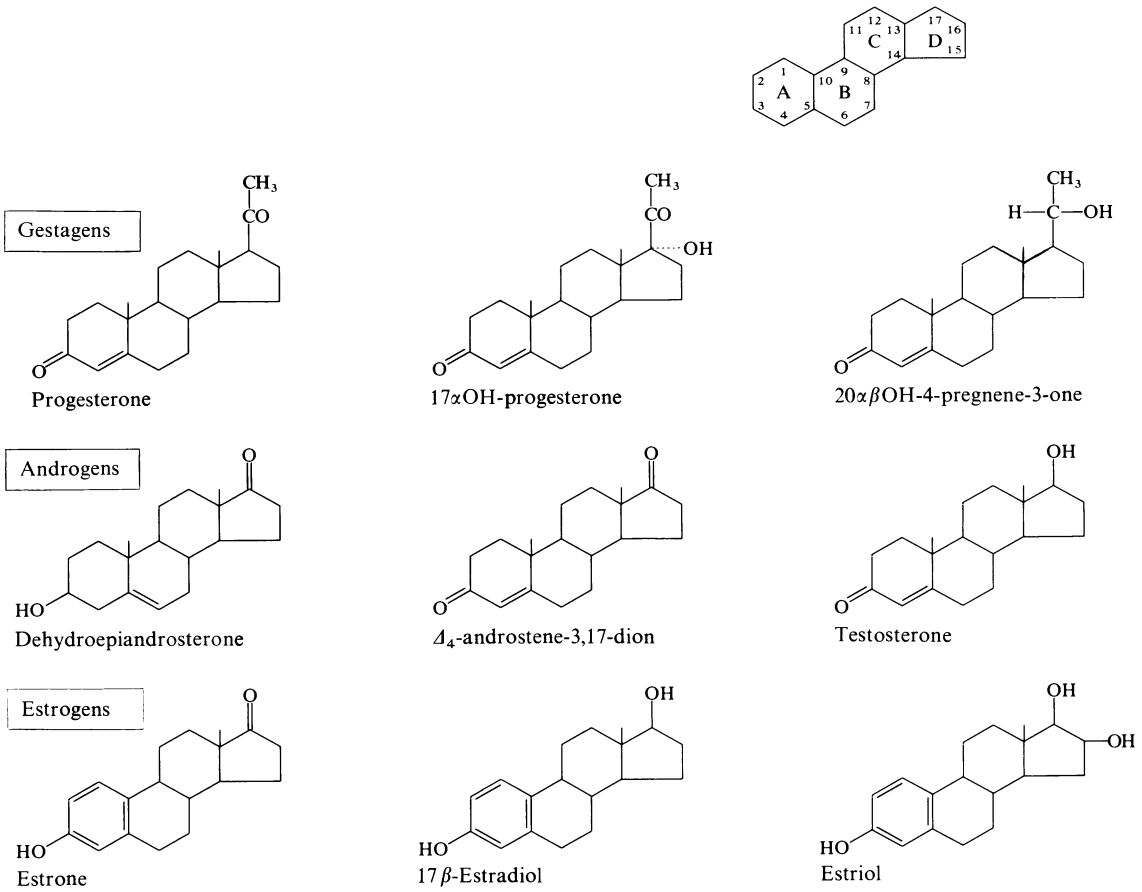
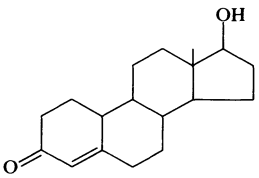
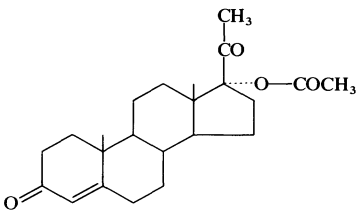
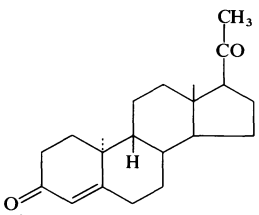


Fig. 8. Ovarian steroid hormones

Table 3. Progestagens

	Compound	Commercial preparation	Main effect
	17α-methyl-	Orgasteron	Androgenic and anabolic
	17α-ethyl-	Nilevar	Anabolic
	17α-ethinyl-	Primolut N; Norlutin	Inhibition of ovulation and gestagenic effect
	-acetate	Primolut Nor; Norlutat	
	-diacetate	with EEME: Metrulen; Ovulen	
	-3 desoxy-	Lynoestrenol	
	-$\Delta^{5,10}$-	with EEME: Enovid	
	-13-ethyl-	with EE: Eugynon, Stediril	
19-nortestosterone derivatives	17α-allyl-3 desoxy-	Gestanon	Protection of pregnancy
	6α-methyl	Provera	Inhibition of ovulation and gestagenic effect
	$\Delta^{6,7}$-6α-methyl-	with EE: Planovin; Volidan	
	$\Delta^{6,7}$-6α-chloro-	with EEME: Sequens	
	3 desoxy-6α-methyl-	with EEME: Neonovum	
17α-acetoxyprogesterone derivatives			
	9β,10α,Δ^4-pregnene-3,20-dione	Duphaston	Gestagenic
Retroprogesterone			

EE = ethinyl estradiol; EEME = mestranol.

Gestagens. Progesterone, 17α-hydroxyprogesterone and 20α,β-hydroxyprogesterone are structurally C_{21} steroids, with a methyl group at C_{10} and C_{13} and a $COCH_3$ side chain at C_{17}. They are fat-soluble and insoluble in diluted alcohol and petroleum ether.

Synthetic gestagens are termed as pre- or progestagens. Gestagens and pregestagens form the group of *progestins*. Progestagen is chemically a derivative of 19-nortestosterone, 17α-hydroxyprogesterone or retroprogesterone. Table 3 shows the progestagens which are currently most important but is by no means complete. The name of a commercial preparation may vary from country to country.

Androgens. Dehydro-epiandrosterone, androstenedione and testosterone have 19 C atoms, with methyl groups at C_{10} and C_{13}.

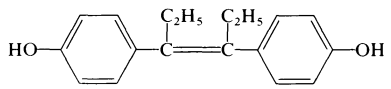
Estrogens. Estrogens are steroids with 18 C atoms and no methyl group at C_{10}. They possess an OH group at C_3, which has a weakly acidic reaction. They also all have a cyclic A ring. They are soluble in oil, alcohol, acetone, chloroform and other fat-soluble substances, and also in alkaline aqueous media. In addition to the three *classic estrogens* (Fig. 8) estrone (E_1), estradiol (E_2) and estriol (E_3) 16 other estrogenic substances have so far been demonstrated in the urine of nonpregnant women (Table 4).

Table 4. Estrogens demonstrable in the urine of woman

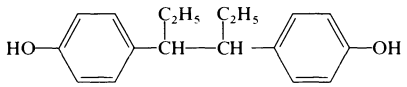
Estrone
16α-hydroxy-estrone, 16β-hydroxy-estrone, 16-keto-estrone, 6ξ-hydroxy-estrone, 2-methoxy-estrone, 18-hydroxy-estrone
17β-estradiol
16-keto-17β-estradiol, 2-methoxy-estradiol, 11β-hydroxy-17β-estradiol
Estriol
16-epiestriol, 16,17-epiestriol, 17-epiestriol, 2-methoxyestriol
Equilenin, estrandiol A and B

These are metabolites of the three genuine ovarian estrogens, estrone, estradiol and estriol.

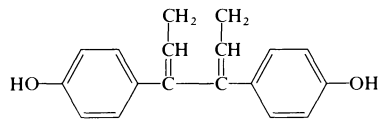
With the exception of 16-epiestriol, the amounts of these other substances are insignificant. We know nothing of their biological activity. In addition to the estrogens occurring naturally in man, animals and botanical organisms, there is a series of synthetic or "artificial" hormones with estrogen activity. The best-known synthetic estrogens are the *stilbenes*: diethylstilbestrol (stilbestrol), dihydroxystilbestrol (hexestrol), dienestrol and tri-p-anisylchlorethylene (Tace) (Fig. 9). These substances are not steroid hormones, and in contrast to natural estrogens are not decomposed in the liver. They are thus effective when taken orally. This is also true of the synthetic estrogen methallenestril (Vallestril).



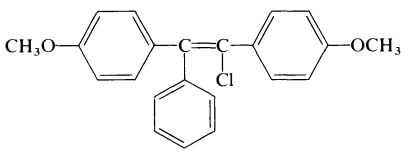
Diethylstilbestrol



Hexestrol



Dienestrol

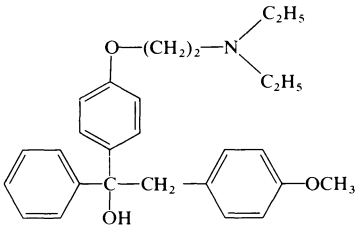


Chlorotrianisene (Tace)

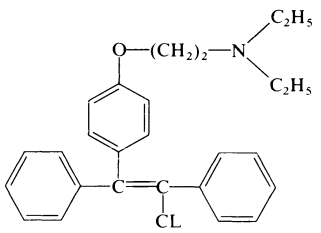
Fig. 9. Structure formulas of the stilbenes

Estrogen antagonists are chemically related to the stilbenes (CALLANTINE, 1967) (Fig. 10). These antagonists are compounds which inhibit certain actions of estrogens. However they also demonstrate a weak estrogenic effect. The most important estrogen antagonists are clomifene (MRL-41) and sexovid (F 6066) (p. 599, 630).

Antiprogestins are substances which prevent the biosynthesis or secretion of progesterone (F 6066, F 6060, methallibur), influence the transport or stability of progesterone in the



Ethamoxytriphetol (MER-25)



Clomiphene (MRL-41)

Fig. 10. Structure formulas of estrogen antagonists

blood (α-1-acid gluco-protein), or interfere with the action of progesterone on the end-organ. The last group of compounds which interfere at the end-organ is large and includes, 17α-ethinyl-4-estren-3,17-diol and its derivatives since they inhibit uterine growth induced by exogenous progesterone. Large doses of estrogens also act as antiprogestins by inhibiting the release of gonadotropins.

2. Biosynthesis

(RICE, 1964, 1966; SAVARD, 1965; RYAN, 1965, 1967; ZANDER, 1963, 1969)

Steroid synthesis occurs in a similar manner in the ovary, testis and adrenal cortex. These glands all have the same embryonal origin from the germinal ridge. Estrogens and androgens are formed in these three endocrine organs, and gestagens in the ovary and adrenal cortex. There are only quantitative differences in the biosynthesis of these steroids in the individual organs, the qualitative nature being identical. The endocrine specificity of these organs is due to the quantitative distribution of the individual steroids secreted. The specificity is based ultimately on the specificity of the enzyme pattern and the ability of this pattern to react to a higher specific controlling mechanism. The "atypical" steroids of single endocrine glands only acquire clinical importance when organic disease leads to a displacement in the quantitative relation of the individual steroids. Typical examples of such cases are the Stein-Leventhal syndrome and adrenogenital syndrome (p. 609 ff.).

There are four morphologically and functionally different structures in the ovary which can react to gonadotropic stimulation and produce sexual steroids. These are the follicle, the corpus luteum, the stroma and hilus cells. The follicle and corpus luteum are predominantly dynamic structures whose activity and morphology change in relation to the age of the woman, the phase of sexual maturity and the development of pathologic processes. The reproductive and endocrine functions of the ovary are related to the morphological and functional integrity of the follicle and corpus luteum. Ascorbic acid is present in large quantities in the ovary and appears to exert a catalytic function there.

Insight into the biosynthesis of the ovarian steroids has been gained by incubation of ovarian homogenates and sections, and by perfusion of the ovaries with labeled precursors of the steroids and with the steroids themselves. Knowledge of the synthesis of ovarian steroids is essential for an understanding of the clinical features of certain endocrine disorders (Fig. 11).

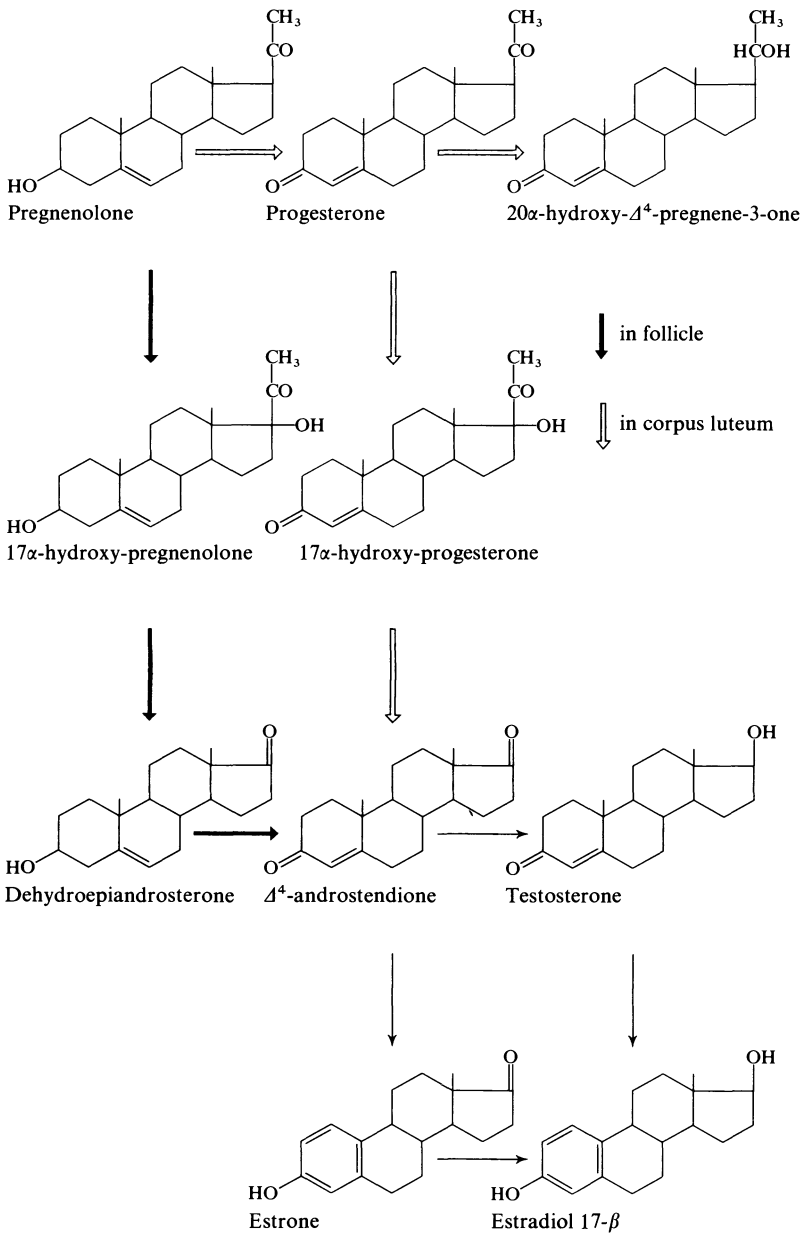


Fig. 11. Biosynthesis of ovarian steroids

a) Biosynthesis of Progesterone

Acetate is the basic material for the formation of steroids in the ovary. It is synthesized to cholesterol via squalene; cholesterol then provides the sterane ring and is converted into the biologically inactive Δ5-pregnenolone, by means of several oxidative intermediate products and by the splitting off of isocapronic acid. Luteinizing hormone and NADPH (reduced nicotineamide-dinucleotide phosphate) are essential for this process. Luteinizing hormone stimulates the 20 and 22 hydrolases and thus provides the stimulus for the splitting off of isocapronic acid. NADPH accelerates the formation of progesterone from pregnenolone by 8–10 times, even in the absence of gonadotropins. Gonadotropins are necessary for the synthesis of pregnenolone, and their absence results in inactivity and consequent atrophy of the ovaries.

It is not yet known whether pituitary gonadotropins still exert a direct influence from this stage on on the biosynthesis of ovarian steroids, or whether further synthesis is determined only by the enzyme pattern and supply of substrate. Oxidation at C 3 by 3β-hydroxy-dehydrogenase and displacement of the double bond to C 3 by $\Delta^{4,5}$-isomerase results in the formation of progesterone. It has been shown that progesterone can be formed from acetate and cholesterol in the follicular wall, corpus luteum and ovarian stroma.

Progesterone has a key position in steroid synthesis, since androgens, estrogens, and glucocorticoids can be formed from it. Because of this, the molecule is extremely labile; it can be rapidly inactivated and transformed (cf. Metabolism, p. 531).

b) Biosynthesis of Androgens
(JUNKMANN, 1960; ZANDER, 1963)

The activity of 17α-hydroxylase is very high in the ovary, and this leads to the oxidative splitting off of the side chain at C 17 via 17α-hydroxyprogesterone, and to the formation of Δ4-androstene-3,17-dione. This route is used predominantly in the corpus luteum.

Androstenedione is not a very potent androgen. Through 17β-hydroxydehydrogenase it is in reversible equilibrium with testosterone, the most potent androgen.

In contrast to the situation in the corpus luteum, in the follicle most of the androstenedione is formed without the intermediary formation of progesterone, via 17-OH-pregnenolone and dehydro-epiandrosterone. 17α-OH-pregnenolone and 17α-OH-progesterone in particular are partly metabolized to pregnanetriol (Fig. 11a) and are excreted in the urine (1–2 mg per day during a normal cycle).

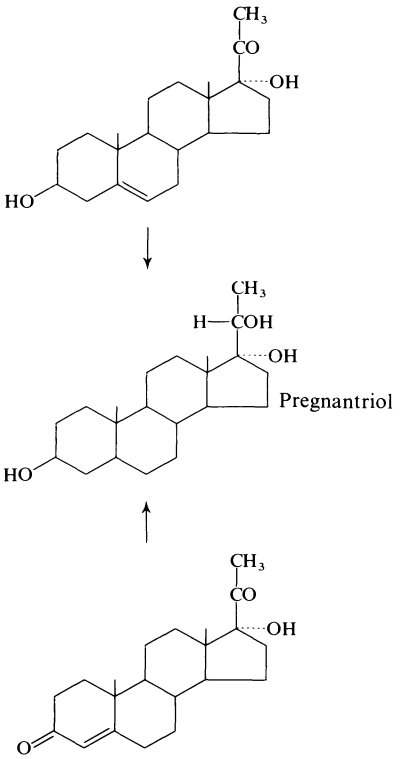


Fig. 11a. Pregnantriol synthesis

Pregnanetriol excretion is greatly increased in the adrenogenital syndrome, due to the failure of enzymatic conversion of 17α-OH-progesterone into 11-desoxy-17-OH-corticosterone.

The two androgens, dehydro-epiandrosterone and androstenedione are released into the blood of a normally menstruating woman, and are partly converted there into testosterone. Increased amounts of these two androgens are released with polycystic ovaries (p. 611).

In contrast to relations in the adrenal cortex, 11β- and 21α-hydroxylase are only weakly active in the ovary, and therefore only small amounts of desoxycorticosterone are formed in the ovary.

c) Biosynthesis of Estrogens

It has been shown that androgens are converted into estrogens in the follicle, corpus luteum, and ovarian stroma. This biosynthesis is similar to that occurring in the testis, adrenal cortex, placenta and liver. In contrast to the situation in the placenta, the androgens in the ovary do not originate from the adrenal cortex, but

are synthesized in the ovary itself. Conversion of the C_{19} steroids, androstenedione and testosterone, into C_{18} phenols probably occurs via their 19-OH and 19-oxy derivatives with subsequent aromatization of the A ring in the microsomes. Conversion of androgens into estrogens is characteristically disturbed in the polycystic ovary (p. 611).

It is assumed that all 3 classic estrogens are formed in the ovary, estradiol being the main estrogen found in follicular fluid and venous ovarian blood. Although it has been shown that there is 16-hydroxylase in the ovary, it is still not known whether estriol is formed via 16-hydroxylation of estradiol or estrone, or whether, as in the fetus, it is formed from hydroxylized androgens. Route and end product of the biosynthesis are dependent on the enzyme pattern of the ovarian structures involved. Absence or reduction of enzyme activity leads to accumulation of intermediary stages (see polycystic ovaries, p. 609).

3. Production and Plasma Content

The amount of sexual steroids produced by the ovary varies greatly in the different phases of life. In addition, it is dependent on the cycle during sexual maturity (Tables 5–9).

Estimations of steroids in venous ovarian blood by means of double-isotope dilution methods have provided interesting information on the secretory activity of the ovaries in relation to the cycle phase (RUNNEBAUM, 1965; MIKHAIL, 1967). A series of biologically active steroids can be isolated from venous blood from the ovary as well as from ovarian tissue (ZANDER, 1958, 1959). It was possible to show by these means that the ovary secretes mainly the steroid androstenedione in the pre-ovulatory phase, and that significant amounts of progesterone and 20α-OH-progesterone are secreted in the ovary even before ovulation. ZANDER (1958) was able to show that these two latter substances were formed before ovulation in the

Table 5. Plasma steroids on day 12 of the cycle. (After MIKHAIL, 1967)

Steroid	Plasma (μg/100 ml)		
	V. cubitalis	V. ovarica	
		right	left[a]
Progesterone	0.149	0.393	1.550!
17α-hydroxy-progesterone	<2.000	<2.000	4.437
20α-hydroxy-4-pregnen-3-one	0.276	0.040	0.108
20β-hydroxy-4-pregnen-3-one	0.011	0.015	0.024
Androstendione	0.683	8.520	8.852!
Testosterone	0.083	0.190	0.242
Dehydroepiandrosterone	1.861	3.956	4.236
Estrone	0.038	0.071	0.172
Estradiol	0.125	0.359	1.760

[a] Ovary containing the ripe follicle.

Table 6. Plasma steroids at bilateral ovulation. (After MIKHAIL, 1967)

Steroid	Plasma (μg/100 ml)		
	V. cubitalis	V. ovarica	
		right	left
Progesterone	0.352	11.320	10.320
17α-hydroxy-progesterone	<2.000	2.668	3.175
20α-hydroxy-4-pregnen-3-one	0.153	0.375	0.300
20β-hydroxy-4-pregnen-3-one	0.008	0.012	0.015
Androstendione	0.578	3.472	2.884
Testosterone	0.159	0.233	0.219
Dehydroepiandrosterone	1.990	2.255	2.537
Estrone	0.235	0.119	0.086
Estradiol	0.089	0.496	0.403

Table 7. Plasma steroids on days 3–4 after ovulation. (After MIKHAIL, 1967)

Steroid	Plasma (μg/100 ml)		
	V. cubitalis	V. ovarica	
		left	right[a]
Progesterone	2.130	2.570	47.100
17α-hydroxy-progesterone	<2.000	<2.000	4.050
20α-hydroxy-4-pregnen-3-one	0.314	0.500	1.390
Androstendione	0.533	1.710	1.760
Dehydroepiandrosterone	1.638	2.080	1.680
Estrone	0.039	0.080	0.065
Estradiol	0.058	0.193	0.418

[a] Ovary with Corpora lutea.

Table 8. Plasma steroids 4 years after menopause. (After MIKHAIL, 1967)

Steroid	Plasma (μg/100 ml)		
	V. cubitalis	V. ovarica	
		right	left
Progesterone	0.075	0.182	0.144
17α-hydroxy-progesterone	<1.000	<1.000	<1.000
20α-hydroxy-4-pregnen-3-one	0.316	0.087	0.058
20β-hydroxy-4-pregnen-3-one	–	0.014	0.015
Androstendione	0.212	0.565	0.352
Testosterone	0.112	0.138	0.102
Dehydroepiandrosterone	1.066	0.970	1.012
Estrone	0.820	0.640	0.540
Estradiol	0.046	0.092	–

Table 9. Production, plasma content and estimation of the most significant ovarian hormones

Hormone	Daily production	Production per cycle	Amount per 100 ml plasma and methods of estimation		Amount excreted in 24-hour urine and methods of estimation	
Estrone (E 1)	*Follicular phase* 50–340 µg (BROWN, 1957)		*Follicular phase* 0.038 µg (MIKHAIL, 1967) After ovulation 0.039 µg (MIKHAIL, 1967)	Gas liquid chromatography, radioisosotpe methods	Postmenstrual minimum 2.8 µg (0.2–7.2) Ovulation maximum 20.1 µg (14.8–27.4) Luteal maximum 11.3 µg (5.1–25)	Colorimetry (BROWN, 1955; 1957)
Estradiol (E 2)	*After ovulation* 160–250 µg (BROWN, 1957) *After menopause* 40 µg	4–8 mg (BROWN, 1957)	*Follicular phase* 0.125 µg (MIKHAIL, 1967) *After ovulation* 0.058 µg (MIKHAIL, 1967)	0.01–0.03 µg (SVENDSEN, 1964)	Postmenstrual minimum 0.7 µg (–) Ovulation maximum 7.9 µg (2.8–21.8) Luteal maximum 5.0 µg (2.1–10.6)	*Before menarche* 1st–8th years <1.0 µg *Sexual maturity* 10–60 µg *After menopause* <10 µg (BROWN, 1963)
Estriol (E 3)					Postmenstrual minimum 4.7 µg (1.9–11.4) Ovulation maximum 30.9 µg (8.1–119.0) Luteal maximum 21.1 µg (5.0–89.0)	
Progesterone	*After ovulation* 15–20 mg (OBER, 1957; CAREY, 1963)	~200 mg (OBER, 1954)	*Follicular phase* ~0.1 µg *Before ovulation* 0.4–0.5 µg *After ovulation* ~1.0 µg	Gas liquid chromatography, radioisotope methods (WOOLEVER, 1963; VAN DER MOLEN, 1965; RIONDEL, 1965; MIKHAIL, 1967; ZANDER, 1967, 1969)	**Pregnandiol** *Follicular phase* 1–2 mg *After ovulation* 3.0–7 mg (KLOPPER, 1957; OBER, 1961; FOTHERBY, 1962)	Colorimetry (KLOPPER, 1955)
20α-Hydroxy-progesterone			*Follicular phase* negative *Before and after ovulation* 0.25 µg	(RUNNEBAUM, 1965; MIKHAIL, 1967)	*After menopause* 0.6 mg (0.3–0.9) (LORAINE, 1958)	

mature Graafian follicle. It has also been shown that 17β-estradiol is the estrogen produced in the largest amounts by the ovary, and that the ovary containing the mature follicle produces significantly greater amounts of estradiol. On the other hand, estradiol secretion has not been definitely demonstrated in the ovaries of post-menopausal women (MIKHAIL, 1967).

Ovarian steroids are probably formed in all cellular structures of the ovary. There is a series of indications implying that the 3 natural gestagens, progesterone, 17α-hydroxyprogesterone and 20α,β-hydroxy-4-pregnene-3-one are synthesized predominantly in the granulosa lutein cells (SHORT, 1964), whereas ovarian androgens are synthesized in the hilus cells, and under normal conditions only to a slight extent (RYAN, 1967). Experiments have been performed with physically separated granulosa and theca cells in the anterior chamber of the eye in rabbits (RYAN, 1966). From the results obtained from these experiments it may be assumed that estriol, estrone and estradiol are formed mainly in the theca cells of the maturing follicle, in the theca-lutein cells of the corpus luteum, and in the theca formations of the stroma (Table 10). All other estrogens are metabolites of the 3 classic estrogens. 17β-estradiol is the main estrogen secreted and formed in the ovary. A continuous secretion of estrogens independent

on the cycle is attributed to the theca formations of the stroma *(basal estrogens)*. This secretion is extremely small in normal ovaries. During the postmenopause and in the case of non-endocrine ovarian tumors, there may be increased estrogen production caused by *cortical stroma- and theca-cell hyperplasia* (LAJOS, 1963).

Table 10. Conversion of pregnenolone and progesterone by the action of granulosa and theca cells isolated from the follicles of woman (RYAN, 1967)

Basic substance	Metabolite synthesized *in vitro*	
	Granulosa	Theca
Pregnenolone	Progesterone 17α-hydroxy-progesterone	Progesterone 17α-hydroxy-progesterone Androstendione Estrone Estradiol
Progesterone	17α-hydroxy-progesterone	17α-hydroxy-progesterone Androstendione Estrone Estradiol

Table 11. Steroids synthesized from acetate in the follicle of woman (RYAN, 1967)

Steroid synthesized	Amount synthesized as a percentage of amount of estradiol (= 100%)
Estradiol	100
Estrone	74
Androstendione	30
Dehydroepiandrosterone	29
Pregnenolone	16
17α-hydroxyprogesterone	8.3
17α-hydroxypregnenolone	3.9
Progesterone	1.5

Without the ovarian structures, small amounts of estrogens are also formed in the zona reticularis of the adrenal cortex *(residual estrogens)*, the Leydig cells of the testis and in great quantity in the syncytiotrophoblast of the placenta. As is to be expected, the secretion of residual estrogens increases in response to stimulation with ACTH.

The *daily secretion of estrogens* by the ovary is 50–340 μg, depending on the phase of the cycle (BROWN, 1957; ZANDER, 1959). The lowest amounts are secreted during menstruation. The maximum (ovulation maximum) is reached at the time of ovulation, and a second, somewhat lower but broader peak (luteal maximum) arises during the luteal phase (Fig. 12), which can, however, fail to occur in women even though ovulation has taken place. Because of the difficulty of the determining the exact time of ovulation (p. 565), the time relation between ovulation and the maximum estrogen secretion is still uncertain. A close connection between these two factors has been deduced from the fact that the time interval between the maximum estrogen production and menstruation is quite constant, is independent of the duration of the cycle and is on average 14 days.

Successful artificial inseminations at the time of the estrogen maximum also indicate a temporal concordance (BROWN, 1962). The total estrogen production during a normal cycle is estimated to be 4–8 mg (BROWN, 1957). It is thought that 10–40 μg of estrone are secreted daily by the adrenals. This amount is almost the same as the amount of estrogens formed during the postmenopause (residual estrogens) (Table 9, Fig. 13).

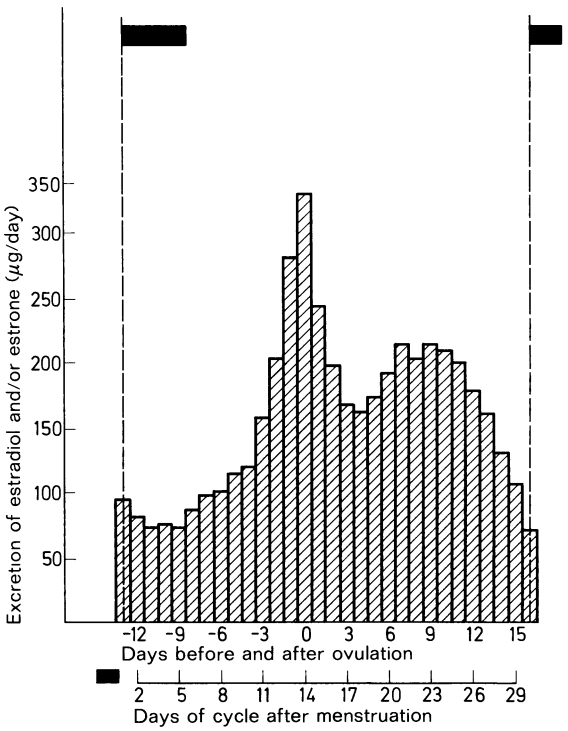


Fig. 12. Excretion of estrogens (estradiol and/or estrone) in an ovulatory cycle (BROWN, 1957). Calculated on the basis of average estrogen excretion from 11 normal cycles

The estrogen level in the plasma is very low, 0.01–0.12 μg per 100 ml plasma, depending on the phase of the cycle.

The formation and secretion of progesterone and 20α-hydroxyprogesterone are also cyclic (Figs. 14 and 15), and start 2–3 days before ovulation or the rise of the basal body temperature (ZANDER, 1958; RUNNEBAUM, 1965; MIKHAIL, 1967). The concentration of these two compounds is many times higher in ovarian

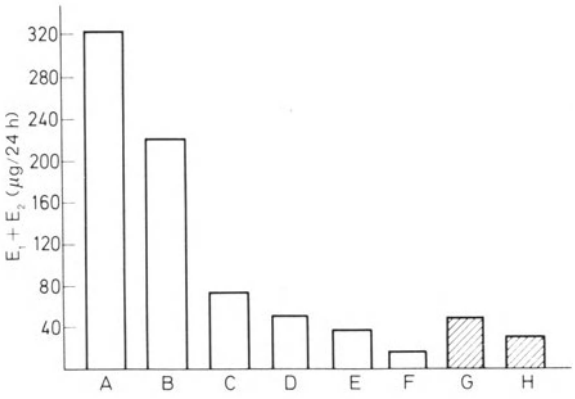


Fig. 13. Daily estrogen excretion in the nonpregnant woman under various conditions (after DICZFALUSY, 1961). *A* ovulation maximum, *B* luteal maximum, *C* menstruation minimum, *D* after X-ray induction of sterility (before the menopause), *E* after bilateral oophorectomy (before the menopause), *F* after bilateral oophorectomy and adrenalectomy, *G* after the menopause, *H* after cortisone medication after the menopause

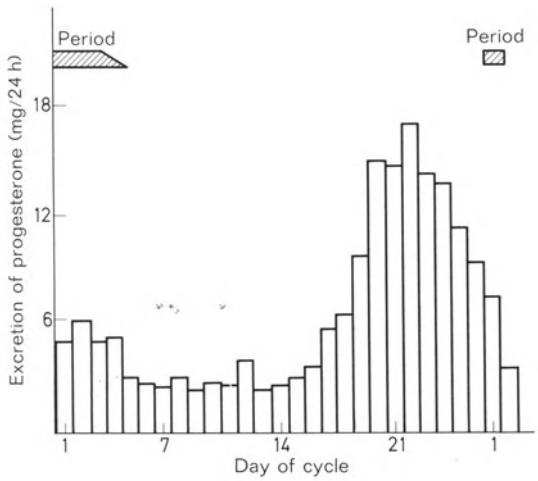


Fig. 14. Progesterone synthesis during the normal biphasic cycle. (After CAREY, 1963)

venous blood than in peripheral blood (Tables 5–7). This shows that they are secreted by the ovaries. The LH peak in the plasma seems to precede the progesterone rise (NEILL, 1967). The total amount of progesterone formed during a cycle is about 200 mg (OBER, 1954). Smaller amounts of 5–8 mg per day are secreted regardless of the stage of the cycle by the adrenals (so-called residual progesterone). The plasma concentration is about 0.1 µg/100 ml in the preovulatory phase, increases rapidly before ovulation and is about 1 µg/100 ml during the luteal phase (WOOLEVER, 1963; RIONDEL, 1965; VAN DER MOLEN, 1965; MIKHAIL, 1967; ZANDER, 1967, 1968). The second gestagen, 20α-hydroxyprogesterone, which is biologically considerably less active, is not demonstrable in the plasma during the follicular phase. Its concentration in the plasma rises rapidly shortly before ovulation and is about 0.25 µg% after ovulation (RUNNEBAUM, 1965; MIKHAIL, 1967). The plasma level of the biologically inactive 17α-hydroxyprogesterone is roughly the same before and after ovulation (MIKHAIL, 1967).

It is not known what amounts of androgens are normally formed in the woman. A small proportion is derived from the ovaries. Androstenedione and dehydro-epiandrosterone are normally secreted from the ovary into the blood and are excreted as 17-ketosteroids in the urine. Some parts is also converted to testosterone in the blood of the normal woman. The plasma level of testosterone is 0.036 ± 0.009 µg/100 ml in sexually mature women (LLOYD, 1966). The level of testosterone is decidedly higher in the plasma in cases of polycystic ovaries, which is in keeping with the increased formation of androstenedione (p. 613, Table 52). About two-thirds of the estrogens circulating in the plasma are bound to β-globulins. Only one-third is in free from in the blood, mainly esterized. The gestagens circulate loosely bound to albumins. We still do not know to

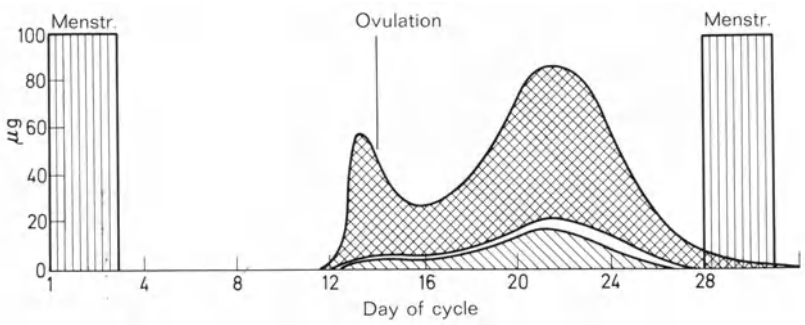


Fig. 15. Total amounts of progesterone (double cross-hatched area) and 20α,β-hydroxy-pregn-4-ene-3-one (single cross-hatched area) in 7 follicles and 32 corpora lutea during the cycle. (After ZANDER, 1958)

what extent the large amounts of enzymes present in the blood are involved in the conversion of estrogens. A conversion of this kind is likely, by analogy with androgens, where 39–54% of testosterone in the blood is derived from androstenedione.

4. Metabolism and Excretion

Valuable information on the metabolism of the ovarian hormones has been obtained by injection of radioactive-labeled sexual steroids.

Following a general principle of hormonal action, estrogens secreted into the blood rapidly disappear into the tissues or are inactivated by the kidneys and liver and excreted in the gut. The half-life of circulating estrogens is only 6 min (PEARLMAN, 1957). Only a small proportion of the estrogens become bound to the specific receptor molecule of organs sensitive to estrogens and produce their specific effect there. There is no accumulation in the end-organs. The highest concentrations are found in the sites of breakdown and excretion, the liver and kidneys.

As deduced from animal experiments, the ovaries and other reproductive organs have no effect on estrogen metabolism. Inactivation of estrogens secreted into the blood occurs by at least two routes: conjugation and oxidation. About half the estrogens secreted are made water-soluble in the liver by esterification with glucuronic and to a lesser degree with sulfuric acid (conjugation), and are then excreted as sodium salts through the kidneys. Another portion is converted into a biologically less active form through oxidation in the liver, is conjugated and also excreted in the urine. About 80% of the estrogens formed leave the organism via the kidneys (BEER, 1955). Investigations on patients with biliary fistula have shown that a fraction of the conjugated estrogens or metabolites reaches the gut with the bile, where it is partly reabsorbed.

This results in an *enterohepatic circulation of estrogens*. Only 7–10% of estrogens are excreted in the free form in the feces. This circulation is probably responsible for the relatively slow excretion of estrogen. The maximum excretion is reached after 24 hours. Only 80% of exogenous estrogens administered are excreted in the urine after 120 hours, while excretion through the gut takes place even more slowly; small amounts of estrogens leave the body in the expired air and sweat and by epithelial desquamation. It is still controversial whether, as in the cases of the synthetic estrogen, tri-p-chlorethylene (Tace), and progesterone, natural estrogens are also stored in adipose tissue. It is assumed that a certain amount of estrogens is stored in adipose tissue, and the greater incidence of carcinoma of the body of the uterus in overweight women has been related to a depot action (Twombly).

Liver cirrhosis, infectious hepatitis and chronic liver diseases can lead to hepatogenic hyperestrogenism (congestion of follicle hormones). This may then provoke dysfunctional bleeding in the woman and feminization (gynecomastia, "abdominal baldness") in the male.

Results of experiments with animals and of tissue analysis and excretion tests in the woman have contributed to our knowledge of the quantitative relations in production, metabolism, and excretion of the sexual hormones. These results, however, vary very widely according to the species and method used. For example, if labeled estradiol is injected 80% of the radioactive material can be regained from the urine. On the other hand, quantitative chemical estimations in the urine show an increase of only 30% of estrogens in the form of estrone, estriol and estradiol. Older methods give a yield of only 10%.

BROWN (1957) found a total of 9–23% (mean 16%) of the 17β-estradiol or estriol given intramuscularly in the urine, 6.8% in the form of estrone, 2% as estradiol and 7.2% as estriol. The estriol excretion was found to be somewhat delayed (so-called estriol lag).

Assuming that administered exogenous estrogens are excreted in the same way as those formed in the organism, the amounts of estrogens formed in the body can be roughly calculated from this excretion estimation by multiplying the amount excreted by 6 (= 100/16). The secretion rate of estrone, estradiol and estriol calculated in this way is 15–314 μg daily, depending on the phase of the cycle. The estrogen excretion in the 24-hour urine is less than 10 μg before sexual maturity is attained and after the menopause. The estrogens in these periods are mainly adrenal estrogens (residual estrogens). Excretion rises shortly before the onset of menarche and reaches adult values as soon as the biphasic cycle is established.

The estrogen excretion is subject to cyclic variations related to the ovarian secretion in sexually mature nonpregnant women (Table 9).

The mean value of the total estrogen excretion in the urine in 24 hours lies between 10 and 60 μg. The estriol, estrone + estradiol quotient is approx. 1 in sexually mature women and the ratio of estrone to estradiol excretion is about 2 : 1. Estrogen excretion is at its lowest during menstruation, beginning to rise on the 7th day of a 28-day cycle and reaching a maximum on the 13th day (ovulation maximum).

BROWN (1955) found an ovulation maximum of 28–99 µg in 24 hours on the 13th day of 10 normal 28-day cycles in eight women between the ages of 17 and 40 (average 56 µg/24 h). The excretion then falls steeply, and rises again parallel to the vascularization of the corpus luteum, reaching a 2nd maximum with the luteal phase (18th to 23rd day) (luteal maximum). There is another fall in excretion 2–3 days before the onset of menstruation (Fig. 16). Bleeding due to withdrawal of estrogens then occurs. The excretion peak of estriol lags 24 hours behind the secretion peak (estriol lag). In the absence of ovulation with endocrine-active ovaries, estrogen production and excretion can remain more or less constant over a long period of time but can also show periodic variations. The daily excretion values are over 10 µg and may even reach values between 80–100 µg/24 h when excretion fluctuates. The endometrium is subjected to the whole spectrum of estrogen stimulation, and corresponding to estrogen production demonstrates all stages, ranging from poor proliferation right up to glandular cystic hyperplasia. The duration of amenorrhea is dependent on the degree of estrogen secretion and the type of secretion increase. In general, the amenorrhea lasts longer as the level is lower and the rise occurs more slowly. It has been shown that the level of estrogen excretion is of prognostic value in amenorrhea (BROWN, 1959). If the total estrogen excretion is repeatedly found to be less than 5 µg/24 h ovarian function is deficient, and the patient will remain amenorrheic. It is uncertain whether a spontaneous cycle can be attained when values are between 5 and 10 µg in 24 hours. The prognosis of a spontaneous cycle is favorable only if the excretion is more than 10 µg/d.

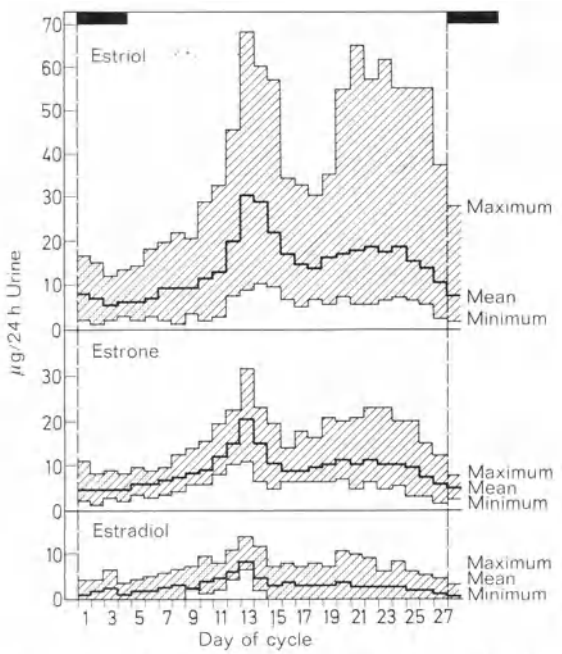


Fig. 16. Excretion of estrone, estradiol, and estriol during the cycle (BROWN, 1959). Mean, minimum and maximum during the cycle in 16 normally menstruating women aged 18–41

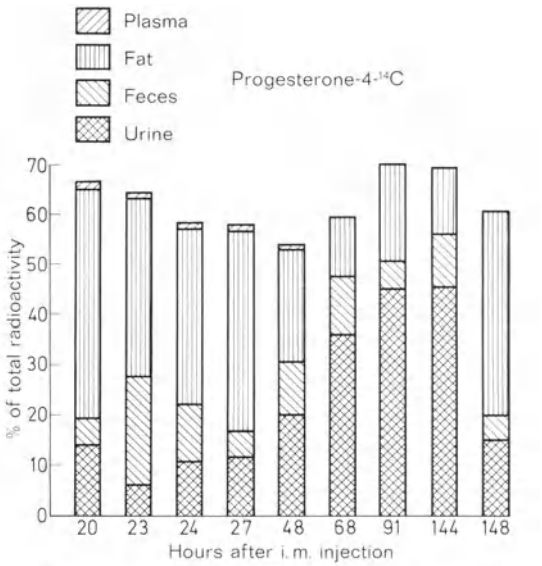


Fig. 17. Distribution of labeled progesterone in the organism. (After PLOTZ, 1959)

Like estrogens, progesterone also disappears rapidly from the blood. The half-life of progesterone circulating in the blood is only 2 min (PEARLMAN, 1957). When progesterone is given intravenously, only 2% of it is present in the blood in the free form after 25 min, and less than 6% in the inactivated conjugated form (PEARLMAN, 1957). If radioactive progesterone is injected intramuscularly, only 1.3% of the administered activity can be demonstrated in the blood at the most, but 80% of the activity is transmitted to the tissue within 3 hours (Fig. 17). Most of this is stored in adipose tissue, while as in the case of estrogens, only minute amounts are found in the end organs. Some of the progesterone secreted is reduced to pregnanediol (a mixture of pregnane-3α, 20α-diol and allopregnane-3α,20α-diol) in the liver, and is excreted in the form of glucuronides mainly via the kidneys and to a lesser extent through the gut and lungs. In the absence of pregnancy, 12–30% of the progesterone produced by the body appears in the urine as sodium pregnanediol glucuronide (KLOPPER, 1956; ZANDER, 1969). From this it can be

calculated that the daily secretion rate of progesterone is 15–20 mg during the luteal phase, and that a total of about 200 mg is excreted during the entire cycle (OBER, 1954). The pregnanediol excretion in the sexually mature woman is related to the cyclic ovarian secretion (Fig. 18). The excretion is 1–2 mg daily during the follicular phase (KLOPPER, 1957; OBER, 1961; FOTHERBY, 1962). This excretion is due to the adrenal progesterone (residual progesterone) and the insignificant amounts of cortexone. Two to three days before ovulation there is a rapid rise in pregnanediol excretion, due to the formation of ovarian progesterone, and 3–7 mg is excreted daily during the luteal phase (KLOPPER, 1957; FOTHERBY, 1962).

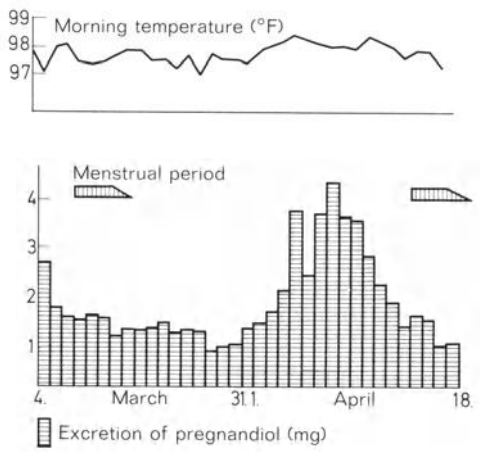


Fig. 18. Pregnandiol excretion and basal temperature curve recorded during a normal cycle. (After KLOPPER, 1957)

Some progesterone is also metabolized by hydroxylation at C_{17} and C_{20} (Fig. 19). The biological activity of progesterone is reduced by conversion to 20α,β-hydroxyprogesterone or 17α-hydroxyprogesterone. The latter substance is an intermediate product in the synthesis of estrogens and androgens, and normally only a small amount is converted to pregnanetriol and excreted as such in the urine in the characteristic cyclic manner. The excretion of this substance precedes that of pregnanediol by about 3 days (FOTHERBY, 1962).

5. Biological Action

The absence and onset of the formation of ovarian hormones, the cyclic secretion and finally their cessation determine the phases

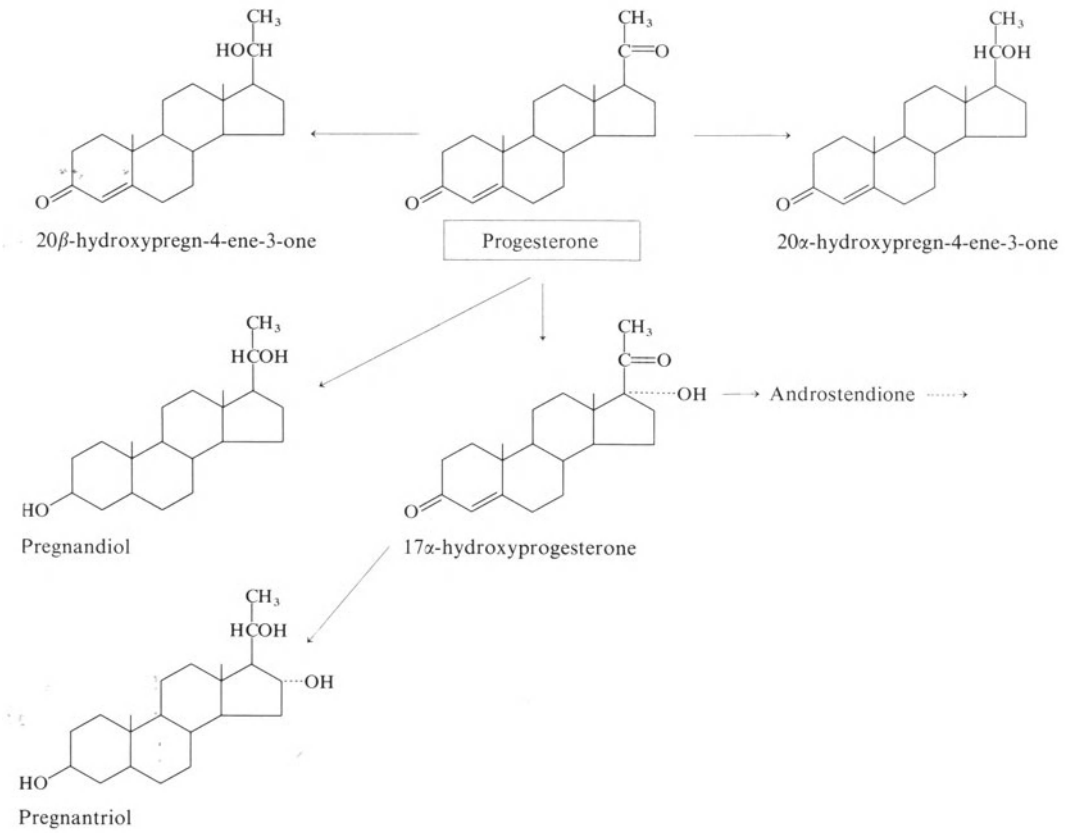


Fig. 19. Pathways of progesterone metabolism

of life in a woman (p. 576). Physical, intellectual and emotional maturity are reached under the action of ovarian hormones. The somatic effect becomes obvious primarily in the reproductive organs, whose development and function are dependent on the presence of estrogens and gestagens. They also cause the development of the secondary sexual characteristics and exert a series of other extragenital effects. In addition they are involved in regulating ovarian endocrine function by their action on gonadotropin secretion (p. 557).

Our knowledge of the physiology of estrogens and gestagens is derived from the state after castration and during ovarian dysfunction and the postmenopause, as well as from the effects of administration of ovarian steroids. Our knowledge about the actions in the woman is modest since in the human, real experimental conditions cannot be produced and there are always endocrine factors producing variable actions. Results from experiments performed mainly in rodents can only be referred with reservations to the conditions in the woman, and there are considerable differences even among the various animal species. Understanding the biological actions of these steroids is, however, essential to the explanation of the pathology and clinical endocrinological picture, as well as to therapeutic use of ovarian hormones.

Both groups of female sexual steroids can be characterized as follows:

Estrogens are substances which produce signs of heat and estrus in castrated female rodents (Allen-Doisy test). They are growth-promoting substances which act primarily on trophism and growth of the reproductive organs. Their action is associated with the presence of folic acid. They increase oxidative phosphorylation in some cells, causing ATP production to be doubled. More amino acids and phosphates are absorbed, and more proteins, ribonucleic acids, glycogen and lipids are synthesized. Thus, estrogens exert a trophotropic-endophylactic or anabolic action on their target organs.

Table 12. Some biological effects of estrogens and gestagens

	Estrogens	Gestagens
Metabolism	Trophotropic and endophylactic:	Ergotropic and dynamogenic:
	Circulation and cell permeability increased; increased deposits of amino acids, carbohydrates, lipids, and phosphates in the cell; increased cell oxidation, augmented synthesis of ATP	Activation of ATPase; increased release of energy
	Retention of sodium and water	Temporary increase in sodium and water excretion
	Growth	Differentiation
	Fall in temperature	Rise in temperature
Vagina	Increased numbers of superficial cells, rise in acidophil and pyknosis indexes; glycogen storage	Massive exfoliation of superficial and intermediary cells (crowding effect)
	pH 3.8–4.5	pH 4.5–5.0
Cervix	Dilatation of ostium uteri and cervical canal	Narrowing of ostium uteri and cervical canal
	Mucus: increased amount (cascade), clear, lower viscosity and increased elasticity (spinnbarkeit), alkaline (pH 7.5–8), penetrable to sperm (Sims-Huhner test)	Mucus: scanty, viscous, cloudy, less penetrable or impenetrable to sperm
	Formation of fern-leaf crystals, increased sugar content (Doyle test)	Test for fern-leaf phenomenon negative
Endometrium	Proliferation:	Secretory transformation:
	Abundant mitoses in glandular epithelia and stroma, multiple layers in glandular epithelia, pronounced increase in alkaline phosphatase	Secretion of glandular epithelia, development of spiral arteries, stromatic edema and pseudodecidual transformation of stroma cells
		Increase in acid phosphatase
		Increased oxygen consumption
Myometrium	Increased intensity and frequency of myometrial activity, increased isometric tension, rise in ATP and actomyosin contents	Relaxation (progesterone block)
	Increased responsiveness to oxytocin	Reduced responsiveness to oxytocin
	Increased circulation	
Ovary	Growth: sensitization to gonadotropins	Reduction of sensitivity to gonadotropins

Various anabolic cellular processes provide the necessary conditions for hypertrophy and hyperplasia of cells. These processes are increased circulation due to liberation of biogenic amines (acetylcholine, histamine, serotonin), increased cellular permeability, and increased cellular oxidation providing biological energy usable in the form of ATP. Apart from influencing the metabolism of proteins, carbohydrates and lipids, estrogens also cause elevated sodium and water retention in the extracellular space. Physiological doses of estrogens exert a permissive action on gestagens i.e. they are necessary for the action of gestagens on the target tissues.

Gestagens preserve pregnancy (implantation and development of gestation). They cause specific differentiation in target structures after the estrogenic growth impulse. They have ergotropic-dynamogenic effects in the reacting organs, and thus promote catabolic cell processes by increasing the release of usable energy by greater activation of ATPase. High doses can inhibit the estrogenic effects in the reacting organs (competitive action). The various actions of gestagens on the reacting organs are described as *progestative actions* (Table 12).

a) Action on the Reproductive Organs

Estrogens cause vascularization, circulation and turgidity to be increased in the *vulva*, and promote growth of the labia minora where the skin becomes thickened. They cause an increase in the secretion of Bartholin's, Skene's and sebaceous glands. Development of the clitoris, labia majora and the pubis are primarily influenced by androgens, and growth of the labial section of the pubis is stimulated partly by estrogens. The *vagina* increases in length and becomes more elastic under the influence of estrogens, and the vaginal vault becomes deeper. The *vaginal epithelium* is highly sensitive to estrogens, more so than the endometrium. It is therefore the classic biological organ for testing estrogens (p. 547). 40 μg daily for 10 days is sufficient to restore atrophic vaginal epithelium in a castrated or elderly woman (Fig. 10, OBER, 1957). Even after 24–48 hours there is an increase in the layers of cells with formation of prekeratin in the superficial layer (Fig. 11 a, OBER). At the same time glycogen is stored from the parabasal zone towards the lumen. This reaction has also been observed in the endometrium and myometrium. Gestagens and androgens, on the other hand, only build up vaginal epithelium to the intermediary layer (Fig. 20). Increased glycogen storage in the vagina of a castrated woman under the influence of progesterone is probably due to partial conversion of progesterone to estrogens. *Vaginal cytology* is used to detect carcinoma and to assess the state of ovarian function (endocrine cytodiagnostic methods, p. 566).

When carried out correctly it is a simple, quick, and reliable method of assessing the levels of estrogens in clinical cases.

It allows the assessment of ovarian function in simple amenorrhea, and a rough estimation of the time of ovulation (p. 566). It is also used to monitor hormone therapy (p. 601) and to examine the potency and duration of action of an estrogen compound (p. 551).

Results obtained by chemical estimation of estrogen excretion and vaginal cytological smears are largely consistent.

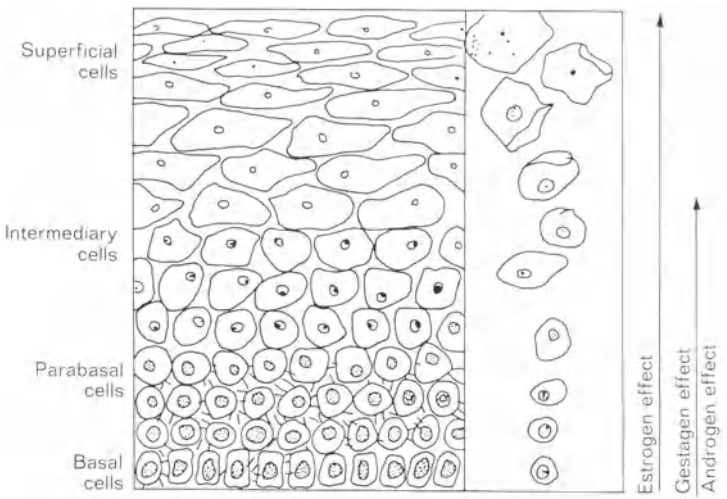


Fig. 20. Structure of the vaginal epithelium in the sexually mature woman, and corresponding exfoliative cells. (After BOSCHANN, 1960)

In order to obtain a perfect smear, a series of conditions are necessary. The cytological smear must be taken before a vaginal examination or treatment. We have found the following *technique for taking* the smear most satisfactory:

A dry self-supporting speculum is introduced and the os uteri is kept in view (Cusco, Trélat speculum). A wooden rod with a small amount of dry cotton wool wrapped round one end is used to take some material from the lateral aspect of the vaginal vault. The cotton wool is then rolled in the opposite direction to that in which it was rotated when the smear was taken to transfer the material to two dry, carefully cleaned microscopic slides. These must be fixed in ether alcohol (96%) for 20–30 min while the smear is still moist and subsequently stained. In children and virgins the smear is taken by means of a glass catheter with a suction balloon. Introduction of a speculum is unnecessary in such cases.

Staining technique (after PAPANICOLAOU, 1942)

1. 80% alcohol ($\frac{1}{2}$ min)
2. 70% alcohol ($\frac{1}{2}$ min)
3. 50% alcohol ($\frac{1}{2}$ min)
4. Distilled water ($\frac{1}{2}$ min)
5. Hemotoxyline (Harris) (3–6 min)
6. Distilled water ($\frac{1}{2}$ min)
7. 0.25% aqueous hydrochloric acid (immerse 6 times)
8. Top water (6 min)
9. Distilled water ($\frac{1}{2}$ min)
10. 50% alcohol ($\frac{1}{2}$ min)
11. 70% alcohol ($\frac{1}{2}$ min)
12. 80% alcohol ($\frac{1}{2}$ min)
13. 95% alcohol ($\frac{1}{2}$ min)
14. Orange G 6 ($1\frac{1}{2}$ min) } separate
16. 95% alcohol ($\frac{1}{2}$ min) } cuvettes
17. EA 50 ($1\frac{1}{2}$ min)
18. 95% alcohol ($1\frac{1}{2}$ min) } separate
19. 95% alcohol ($\frac{1}{2}$ min) } cuvettes
20. 95% alcohol ($\frac{1}{2}$ min) }
21. Absolute alcohol ($1\frac{1}{2}$ min)
22. Xylol alcohol ($\frac{1}{2}$ min)
23. Xylol ($\frac{1}{2}$ min)

If the smear shows inflammatory changes, it is advisable to insert a terramycin capsule (50 mg) deep into the vagina 2 to 3 days before the next smear is taken. Refer to the following authors for the endocrinological evaluation of vaginal cytological smears: PAPANICOLAOU (1033, 1946), SHORT (1940), ROTH (1950), WIED (1953), SCHMITT (1953) and textbooks of cytology by BOSCHANN (1960), KOOS (1961), DE NEEF (1967), PUNDEL (1952, 1957, 1966), SMOLKA (1965) and ZINSER (1957).

Rough quantitative assessments of the levels of estrogens and gestagens are possible with vaginal cytology (PAPANICOLAOU, 1933; PUNDEL, 1952). The *atrophic vaginal smear* encountered before menarche, after the menopause, and in vegetative ovarian insufficiency during sexual maturity shows a predominance of parabasal and basal cells. These cells are small and round, each containing a large, chromatin-rich vesicular nucleus in the basophilic cytoplasm (Fig. 21 colored cytological slides). In addition there are a few larger ovoid intermediary cells, as well as a varying number of leukocytes and bacteria (vaginitis senilis).

In the vaginal cytological picture, an increasing number of large-surfaced, polygonal superficial cells appears under the influence

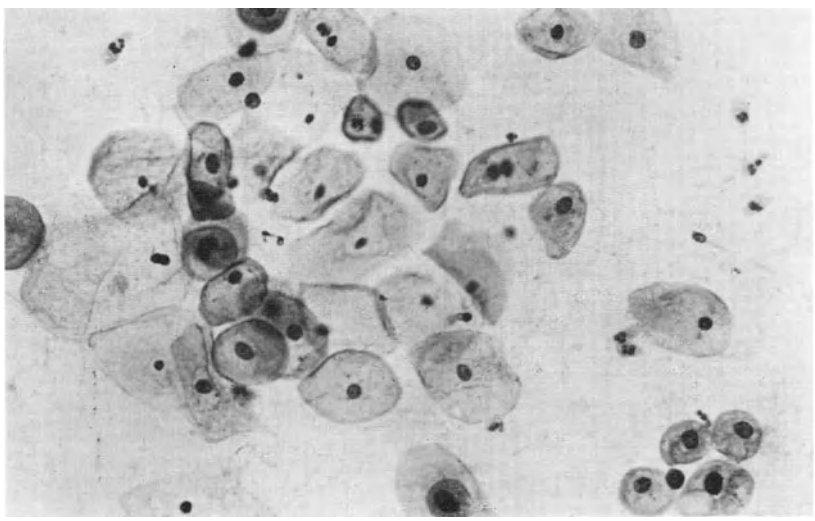


Fig. 21. Atrophic vaginal smear

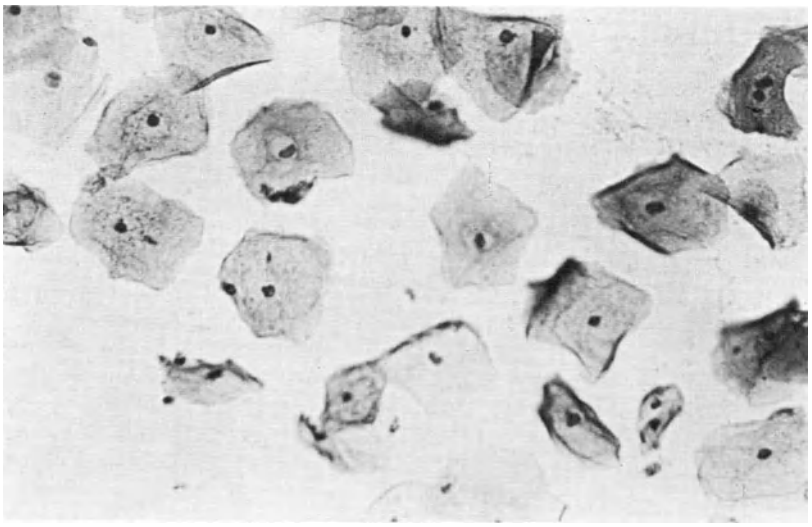


Fig. 22. Preovulatory vaginal smear

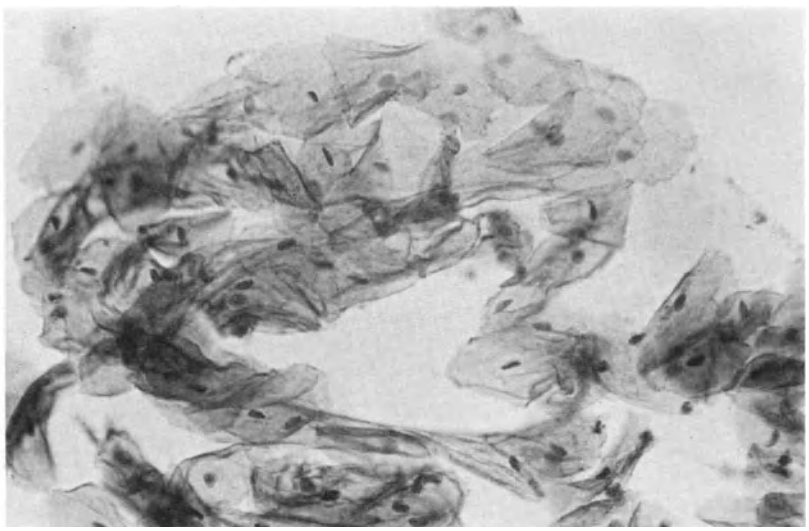


Fig. 23. Luteal vaginal smear

of estrogens. These cells have a pyknotic, structureless nucleus, often associated with a perinuclear chamber, and the cytoplasm is transparent and acidophilic. The cells lie separately and make up 40–80 % of the desquamated cells before ovulation (Fig. 22). Besides these cornified superficial cells, uncornified, karyopyknotic superficial cells are also found during the proliferative phase. These can be distinguished from the cornified cells only by the fact that their cytoplasm shows an affinity for basic stains and assumes a bluish violet color. Intermediary cells originating from the stratum spinosum are rarely seen. They are smaller and ovoid, the nucleus is vesicular and the cytoplasm is basophilic. Basal and parabasal cells do not normally arise in the smears of sexually mature women. The smear also reveals leukocytes, erythrocytes, Döderlein bacteria, fibrin and mucus in varying amounts depending on the cyclic phase.

The *pyknosis index* (= percentage of cells with pyknotic nuclei in the cytological smear) and to a lesser degree the *acidophil index* (= percentage of acidophilic cells) are of practical importance in assessment of the estrogen action in cases of dysfunctional bleeding or amenorrhea, and in the investigation of sterility (Fig. 24). The pyknosis index is simply and quickly determined, and is a reliable indicator of estrogen production. There is a significant relation between the pyknosis index

and the logarithm of estrogen excretion (JOHANNISSON, 1961). Continuous preparation of vaginal smears in association with assessment of the cervical factor permits an approximate estimate of the time of ovulation (p. 567). The pyknosis index is over 40% before ovulation and between 60–80% at the time of ovulation, when leukocytes and Döderlein bacteria almost completely disappear from the picture.

After ovulation progesterone acts on the fully developed vaginal epithelium, causing massive desquamation of the superficial and intermediary cells (so-called luteal effect in the vagina). In the smear (Fig. 23) group formations with superimposed cells in place are seen (crowding effect). There is a predominance of superficial cells and later of intermediary cells with basophilic cytoplasm, which is rolled in at the edges due to loss of cellular turgidity. Only a few superficial cells with karyopyknosis and acidophilia are still found. The pyknosis index falls below 30%.

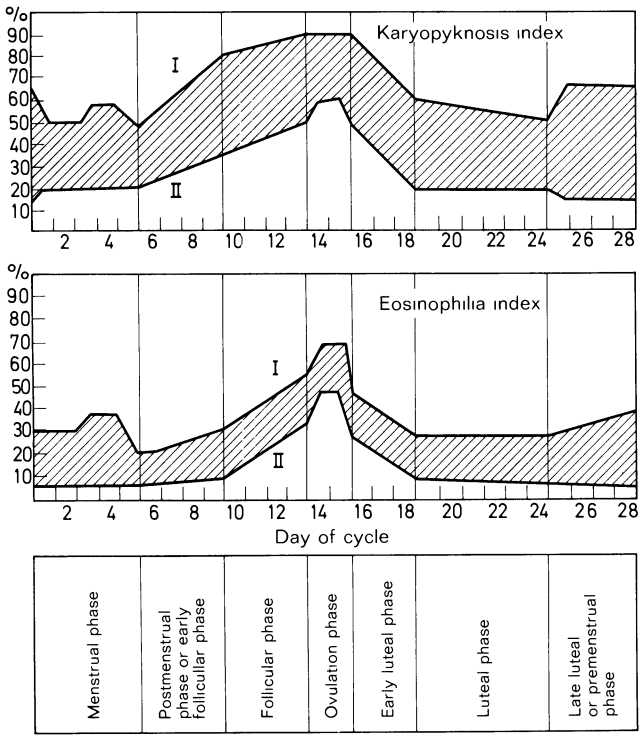


Fig. 24. Changes in the karyopyknosis index and the eosinophilia index over a normal 28-day cycle. Normal range: I = maximum, II = minimum. (After PUNDEL, 1952)

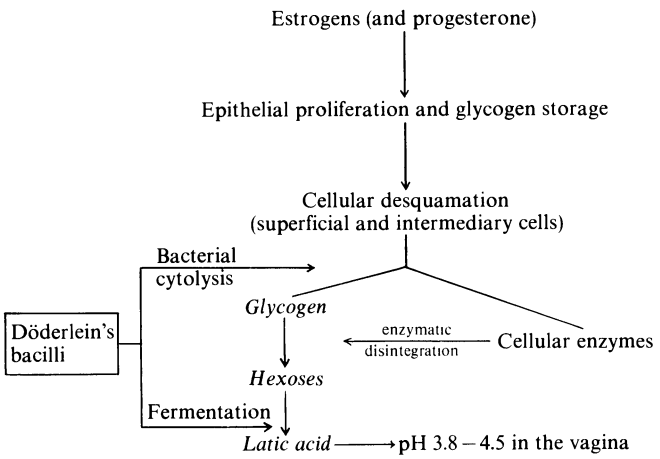


Fig. 25. Lactic acid synthesis (self-purification) in the vagina

Epithelial proliferation, glycogen storage and increased desquamation of the superficial and intermediary cells caused by the actions of estrogens and progesterone are physiological processes of basic importance to the so-called *self-purification of the vagina* (Fig. 25). The glycogen liberated by bacterial cytolysis is converted into hexoses by enzymes contained in the cells. The hexoses are then fermented to lactic acid by the gram-positive Döderlein bacteria, giving pH 3.8–4.5. Only Döderlein bacteria can thrive in these acidic surroundings. Growth of pathogenic organisms is prevented (with the exception of trichomonads).

Epithelial proliferation, glycogen storage and desquamation of vaginal epithelium reach their maximum at the time of ovulation and in the premenstrual phase. The time of these maxima depends on the maximal secretions of the sexual steroids. Thus, glycolysis and lactic acid formation due to Döderlein bacteria are most pronounced between ovulation and menstruation. pH values are lowest (3.8–4) at this time, rising to 5 during the other phases of the cycle. The lowest values are found in the anterior vaginal vault, the highest at the introitus. Vaginal acidity is of physiological importance since most pathogenic organisms cannot thrive at a pH between 4.1 and 4.9. Menstrual blood almost completely neutralizes the pH, and there is an increased risk of infection during menstruation. Similar conditions are found during the postmenopause and in prepuberty, when the pH is 7 and 6 respectively.

The infantile uterus increases in size (length increases from 4 to 8 cm), becomes more vascular and increases from 16 to 60 g in weight under the trophic influence of estrogens. The length of the cervix is greater than that of the body of the uterus during infancy. The final ratio of the fundus to the cervix in the sexually mature women is 2 : 1 because the cervix does not grow as quickly as the fundus (Fig. 26).

Estrogens cause a trophotrophic-endophylactic reaction in the muscle cells, together with a rise in the ATP concentration and contractile protein (actomyosin). Intensity and frequency of myometrial activity also increase. Progesterone in contrast lowers the frequency of contractions of myometrial cells and their ability to respond to oxytocin (KNAUS, 1953; CSAPO, 1966) (progesterone block after CSAPO, 1961, 1963) by diminishing the concentration of intracellular potassium (CSAPO, 1956).

Characteristic changes occur in the cervix factor in the preovulatory phase 3–4 days before ovulation, due to increased stimulation by estrogens (RAUSCHER, 1954; SABINE, 1941) (Fig. 27). The dilatation of the os uteri is increased (ASPLUND, 1952) (Fig. 28), as is the amount of cervical mucus displaying alkaline reaction (pH 7–8) (VIERGIVER, 1944). The transparency, elasticity, spinnbarkeit (PALMER, 1941; CLIFT, 1945) and penetration capacity of the mucus by sperms are also increased (p. 625, Sims-Huhner test) (SIMS, 1868; HUHNER, 1913). Viscosity (VIERGIVER, 1946) and the leukocytic and bacterial contents are diminished. In addition, characteristic crystallization occurs when the mucus is allowed to dry in the air on a microscope slide, due to the increase in NaCl content. This crystallization takes the form of fern-leaf crystals (fern test or arborization phenomenon) (Fig. 71).

For the *fern test*, a pipette is cleaned with distilled water and then dried; some mucus is then aspirated from the cervical canal with

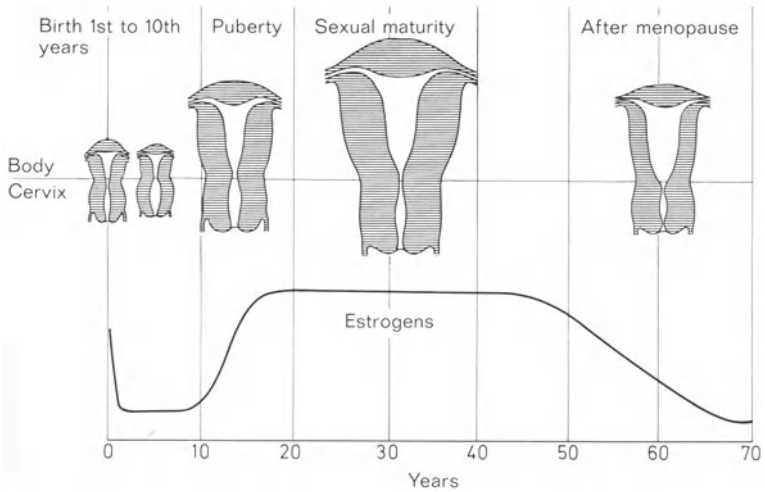


Fig. 26. Relative size of the uterus, body-cervix ratio, and estrogen excretion at different stages of development. (After Pschyrembel, 1968)

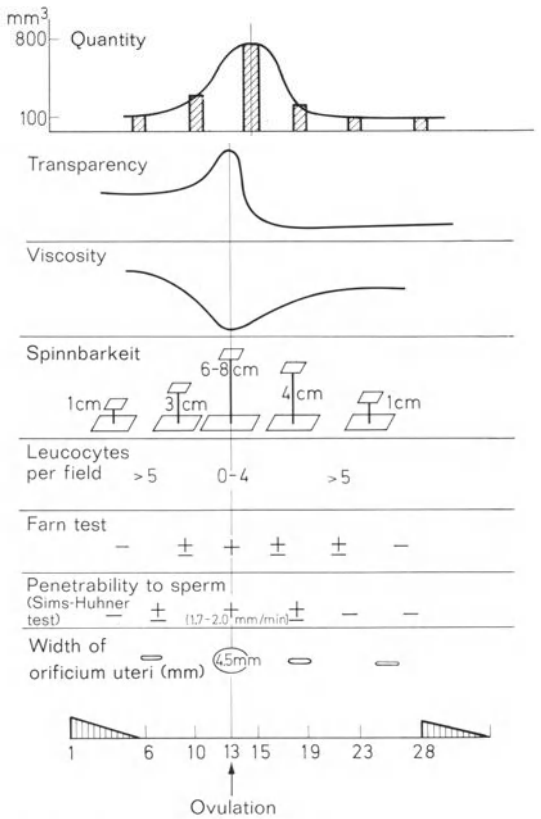
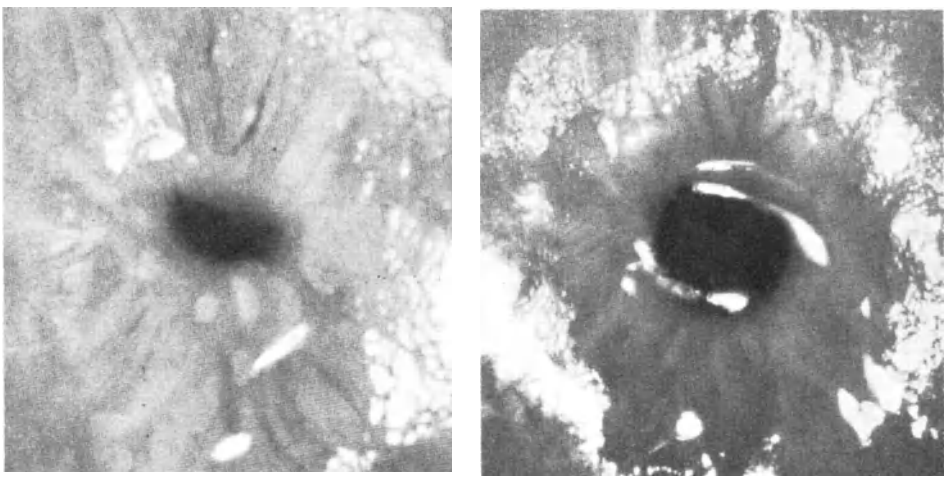


Fig. 27. Cyclic changes in the cervix factor

this pipette and blown out onto two microscope slides. Large amounts of glass-clear mucus (so-called cascade formation) are usually found around the time of ovulation, and fern leaf-like crystals of salt crystallize out from this mucus after 15–20 min (PAPANICOLAOU, 1946; RYDBERG, 1948; CAMPOS DA PAZ, 1953). Assessment is made under 100 times magnification. This phenomenon is quite nonspecific, and can also be observed before ovulation or when other body fluids, e.g. saliva, are allowed to dry up (ZONDEK, 1954). A reaction of this type always occurs when mucopolysaccharides and electrolytes come into contact. In the cervical mucus, it is a sign that estrogen influence is adequate. The fern test becomes positive 24–48 hours after administration of estrogens (PUCK, 1958), and becomes negative 10 days after ovulation has occurred. False-positive results are always obtained when the pipette used for aspiration or the slides are contaminated with salt, or when blood is present in the cervical mucus. The semi-quantitative method of colorimetric estimation of NaCl content in cervical mucus can also be helpful in determination of the time of ovulation (MCSWEENY, 1964). An applicator with a strip of filter paper soaked in silver nitrate and potassium chromate is used for this test. Due to metabolic changes produced in the cervical glands by the influence of estrogens, the glucose content is increased and the acid content reduced, parallel to the increased NaCl content. *Doyle's test* for colorimetric demonstration of glucose content can also be used to determine the time of ovulation (DOYLE, 1959). DOYLE's and the MCSWEENEY's test are of limited practical importance.

The spinnbarkeit of the cervical mucus is at its greatest at the time of ovulation (PALMER, 1941; COHEN, 1952), and is best examined as follows: a forceps is used to pull out a thread of mucus from the cervix and the length of the thread at its breaking point is estimated. Normally this length can reach 8–10 cm shortly before and during ovulation. Inadequate spinn-



a b

Fig. 28. a) Ostium uteri before ovulation (4 days before lowest point of temperature curve). b) Ostium uteri of the same woman in the same cycle at the time of ovulation (day corresponding to lowest point of temperature) (OBER, 1957)

barkeit at this time indicates estrogen deficiency. Progesterone reverses the cervical changes already described: the external os and uterine isthmus become narrower, the amount of cervical mucus decreases to between one eighth and one tenth of the ovulation maximum, the mucus becomes cloudy, yellowish and thick due to increased leukocytic invasion, and it is impenetrable to sperms. The spinnbarkeit progressively diminishes, and the fern-leaf phenomenon is no longer demonstrable 8–10 days after ovulation. The inhibitory effect of progesterone on the arborization phenomenon in the cervical mucus can be shown quantitatively: administration of 25 mg estradiol benzoate to the castrated woman results in the fern-leaf phenomenon, but this is prevented by giving an injection of 15 mg progesterone at the same time (ZONDEK, 1957). Increased function of the cervical glands at the time of ovulation can present clinically as intermenstrual discharge.

Cyclic changes in the endometrium occur at the same time as those in the ovary. The so-called *endometrial cycle* results from the influence of estrogens and gestagens, and the associated changes arise almost exclusively in the functionalis (HITSCHMAN, 1908; SCHRÖDER, 1928); NOYES, 1950; BEHRENS, 1953; BARTELMEZ, 1957; SCHMIDT-MATHIESSEN, 1963). Three functional and histological phases can be differentiated:

1. the proliferative, regenerative, estrogen or preovulatory phase;
2. the secretory, transformative, progesterone or postovulatory phase;
3. the menstrual or desquamation phase.

Glandular tubules and stroma with vessels react characteristically to estrogens and gestagens. The changes are so specific that it is possible to determine the stage of the ovarian cycle to 48 hours from the endometrial changes especially during the luteal phase (Fig. 29). In a normal cycle of 28 days, the menstrual phase lasts 1–4 days, the proliferative phase from days 5–13, and the secretory phase from days 14–28. The duration of the postovulatory phase is frequently constant and is usually 14 ± 2 days (ROCK, 1949), whereas the preovulatory phase is of unlimited duration.

Increasing estrogenic effect causes the *proliferative phase*. From day 5 of the cycle, new functionalis is formed from the basalis, and reaches a mean thickness of 3.5 mm at the time of ovulation. At the same time epithelialization of the uterine wound surface begins, with sprouting of the epithelia of the remaining glands.

The proliferative influence of estrogens can be recognized histologically from the increasing number of mitoses in the glandular epithelia and stroma.

Protein synthesis associated with the building up of the functionalis is reflected in the increased content of ribonucleic acids in the glandular epithelia and the increased mitotic activity by the raised desoxyribonucleic acid content. At the same time, the concentration of alkaline phosphatase increases considerably, reaching its maximum at the time of ovulation.

Three periods can be differentiated histologically: early, middle and late proliferative phases. The originally sparse, narrow, and stretched glandular tubules begin to branch, become wider and show definite meandering at the end of the proliferative phase. The nuclei originally situated basally come to lie at different levels in the cells due to increasing nuclear division. This leads to pseudo layers in the glandular epithelium, which disappear with the mitoses during the early secretory phase. The stroma usually has a dense cellular framework during the proliferative phase, and the initially small number of mitoses increases considerably up to ovulation. A transient edema, which varies widely, leads to a picture of "naked nuclei" in the stroma cells with little cytoplasm. This edema causes rapid thickening of the functionalis from day 10 onwards. Severe extensive stromal edema prevents secretory transformation.

After ovulation, the functionalis remains under the influence of estrogens, but is also affected by increasing amounts of gestagens secreted by the corpus luteum. *Secretory transformation* occurs in the endometrium "prepared" by the estrogens. In the first half of the secretory phase, the combined effect of both hormone groups becomes primarily obvious in the glandular tubules: formation of basal vacuoles due to storage of mucus and glycogen, secretion into the widened tubular lumen, increasing convolutions of the glandular tubules, which gives a saw-like structure to the histological picture during the secretory phase. In the second half of the transformative phase, the influence on the stroma is predominant: development of spiral arteries found only in menstruating primates, forming a subepithelial plexus, extensive stromal edema and pseudodecidual conversion of the stroma cells with formation of perivascular sheaths around the spiral arteries, and development of a superficial compacta and spongiosa lying next to the basalis. Acidic phosphatase and dehydrogenases replace the alkaline phosphatase after ovulation. At the same time, progesterone acts ergotropically and dynamically to increase the activity of adenosine triphosphase and oxygen requirements in the endometrium.

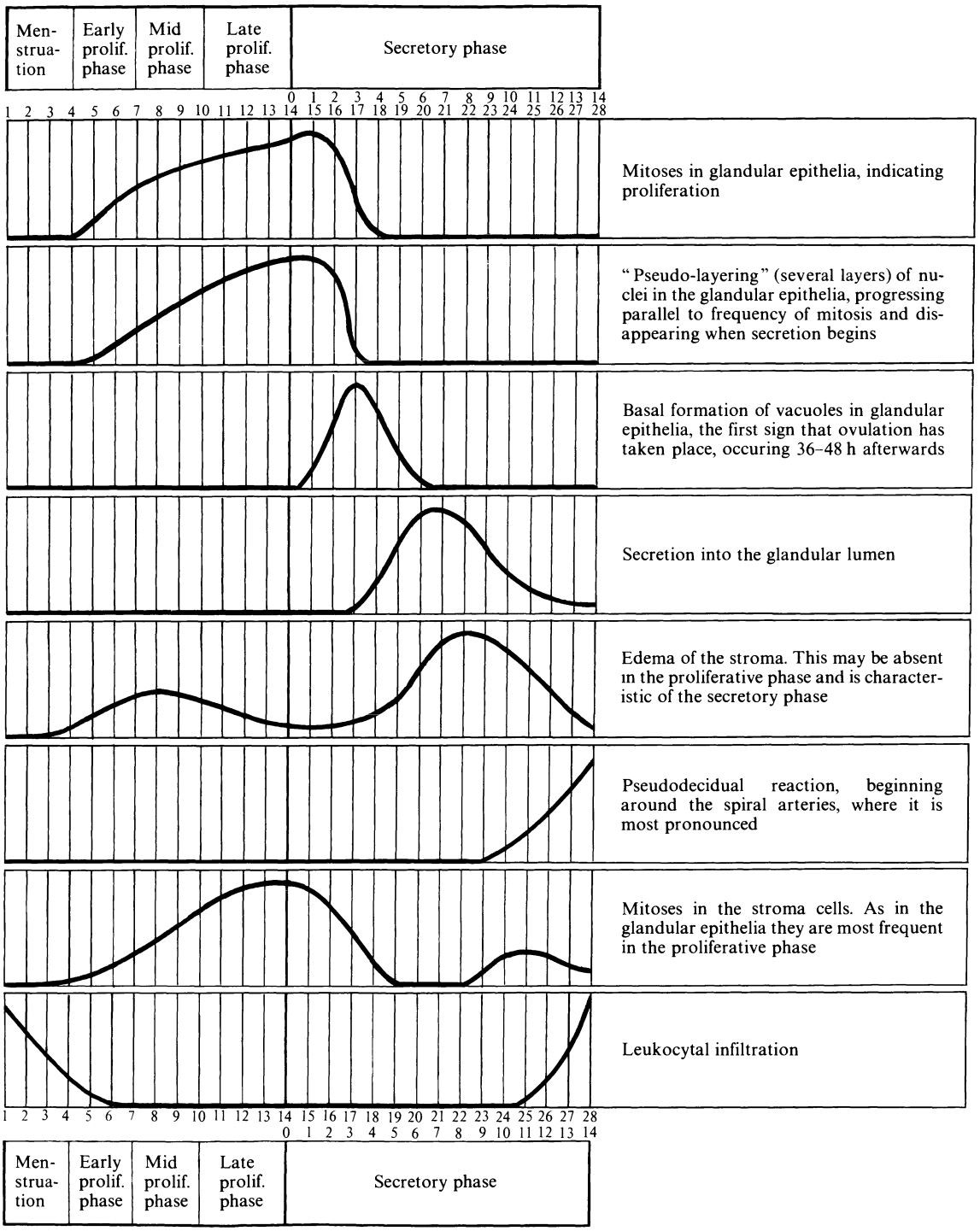


Fig. 29. Determination of the stage in the endometrial cycle. Course of characteristic morphologic signs in the endometrium during the cycle. (After R. W. NOYES, 1950)

The first signs of progesterone influence arise in the basal sections of the glandular epithelium. Vacuoles appear, and the cell nuclei become arranged at the same level in the middle of the cell. Pseudolayers disappear. Mucus and glycogen storage is demonstrable in the cell basis even only 24–48 hours after ovulation. Glycogen secretion into the lumen of the glandular tubules begins on day 4. Glandular tubules continue to become convoluted. At the same time, cell nuclei no longer undergoing mitosis are once more found at the cell basis. A stromal reaction which can be detected histologically arises from day 7 at the time when the blastocyste becomes implanted if fertilization

has occurred. The stroma becomes edematous and loose, mitotic activity of the stroma cells increases again, and some of the cells form a net due to water deposition, while some are converted into decidual cells due to storage of glycogen and lipids (so-called pseudodecidual reaction). Nuclei and cytoplasm become larger, and the pseudodecidual cells arrange themselves in layers of the functionalis. This distinguishes the superficially situated compacta from the spongiosa.

The temporal phases of the various histological changes in the functionalis are shown in Fig. 29 (NOYES, 1950).

If fertilization fails to occur, the corpus luteum regresses as early as 10 days after ovulation due to the absence of the action of chorionic gonadotropin produced by the zygote. The significance of chorionic gonadotropin for the preservation of the corpus luteum (so-called luteotropic action) can be shown experimentally: injections of 5000 IU chorionic gonadotropin on the 3rd, 5th and 7th postovulatory days converts the corpus luteum of menstruation into one of pseudopregnancy. Regression of the corpus luteum results in a fall of the levels of estrogens and gestagens in the blood, which in turn gives rise to regressive changes in the functionalis even from the 4th day before menstruation. There is first lymphocytic and then leukocytic infiltration of the stroma, which shrinks at the same time due to loss of water. As much as 90% of the mucosal volume can be lost through this shrinkage. This has been observed directly in women with visible endometriosis. (HOFFMANN, 1953).

The external course of the associated tissue changes can be observed directly when the technique introduced by MARKEE (1940) is used. Endometrial fragments of a Rhesus monkey are implanted into the anterior chamber of the eye of the same animal. The first sign that menstruation is about to occur is pallor of the vascular regions due to vasoconstriction of the individual spiral arteries. Necrotic foci arise in the compacta, accompanied by discrete hemorrhage. Pressure of the recirculating blood then results in rupture of the arterioles damaged by anoxia. A thin streak of blood is emitted from the opened vessel (bleeding per rhexin). This gives rise to massive hemorrhage into the tissue, causing destruction and desquamation of the tissue with the onset of menstruation. Thus menstruation is a hormone-withdrawal bleeding. It is different from breakthrough bleeding in the anovulatory cycle, in which the relative estrogen deficiency results in bleeding per diapedesim. In this case, the blood is coagulable, since the fibrinolytic enzymes liberated with massive destruction of the endometrium are absent.

The significance of estrogens for endometrial vascularization can be shown experimentally. Intravenous administration of 2 mg estrone or estradiol provokes extensive arterial hyperemia in the endometrium after 3 minutes, and secondary rhythmic vasoconstrictions result from the withdrawal (LOESER, 1948; BURGER, 1957). The original factor responsible for vasoconstriction of the spiral arteries is not known. It is possible that at the beginning of the process an unknown *bleeding factor* is liberated in the endometrium by estrogen and progesterone withdrawal. This factor may be identical to the potent vasoconstricting substance postulated by MARKEE (1950) or to the phytotoxic substance, "menotoxin", thought by MACHT and LUBIN (1924) to arise in damaged endometrium or to the "toxic endometrial euglobulin" of SMITH (1950). Changes in vascularization of the functionalis very probably have a great effect (BARTELMEZ, 1931, 1933, 1937; MARKEE, 1950; SCHLEGEL, 1945/46; DALGAARD, 1946), although the importance of this has not been fully accepted (KAISER, 1948). It is still a subject of controversy whether arteriovenous shunts with contractile elements under nervous control are also involved (SCHLEGEL, 1946). The importance of inadequate lymph drainage (REYNOLDS, 1947) with accumulation of toxic breakdown products of proteins is still open to discussion.

The periodic uterine bleeding is termed *menstruation* (period, monthly bleeding). It is accompanied by desquamation of the functionalis of the endometrium, with rejection of the major parts of the zona compacta and spongiosa. Since the normal endometrial cycle results in secretory transformation of the endometrium, menstruation is usually the result of bleeding from the secretory converted endometrium. It is, however, pointless to attach the clinical term "menstruation" to the presence of a certain histological change which is only exceptionally verified. Ovulation fails to occur in 4–8% of all regularly menstruating women (EFFKEMANN, 1939; ROCK, 1939; SIEGLER, 1951).

It is probable that occasional *anovulatory cycles* also arise during the "best" years of the reproductive period of a woman (Table 13). According to investigations, 3–10% of all cycles are anovular in women aged between

Table 13. Percentage of anovulatory cycles as a function of age in woman. (VOLLMAN in: HARTMAN, 1962)

	Gynecologic age					
	1–2	3–4	5–6	7–8	9–10	11–12
%	55.0	35.0	24.0	14.5	7.3	3.0
Average	17%					

20 and 40 (DÖRING, 1963; MATSUMOTO, 1962). Anovulation is physiological in the first few years after menarche, post partum and in the premenopause (VOLLMANN, Fig. 30, DÖRING), and causes relative sterility during these periods (ASHLEY-MONTAGU, 1946; COOPERMAN, 1949). Depending on the type of groups examined, 3–15% of cycles are anovulatory (FELDING, 1949; OVERSTREET, 1948; COLLETT, 1954; MATSUMOTO, 1962). This is observed much more frequently in patients attending sterility clinics than in other groups (ROCK, 1939). [The reader is also referred to the sections on dysfunctional bleeding, polycystic ovaries, transition periods in the woman.]

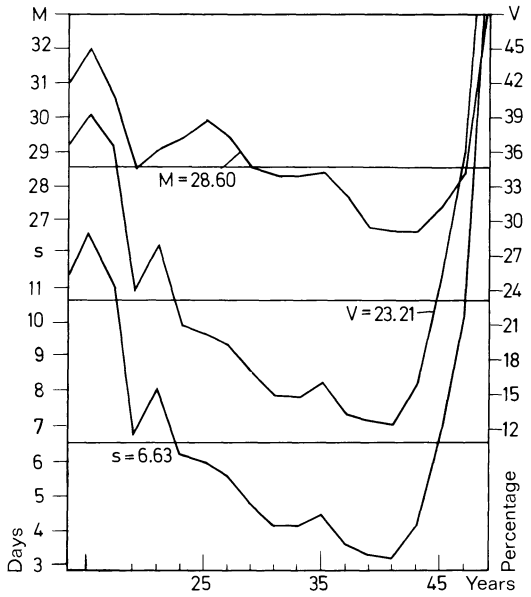


Fig. 30. Dependence of average length of cycle (M = mean = 28.60 days), its variability (V = 23.21 days), and standard deviation (s = 6.63) on age. (After VOLLMAN in HARTMAN, 1962)

Looked at biologically, the *menstrual cycle* begins with the proliferative phase and ends with the termination of menstruation. For practical reasons, however, it has become customary to consider the first day of menstruation as the beginning of the cycle, and the last day before the next menstruation as the end of the cycle. Thus, the *duration* of the cycle, is the interval from the first day of menstruation up to and including the day before the next period. Since ovulatory cycles are very rarely distinguished from anovulatory, WHO has suggested that it would be best to adopt the term "menstrual onset interval". In spite of the varying composition of the groups examined (age distribution, period of observation, race, ovulatory ± anovulatory cycles etc.), the statistical results of different authors about the duration of the cycle show only minor discrepancies. According to different investigations in large groups, this interval is 28.4–30.8 days (KING, 1933; FLUHMANN, 1934; LATZ, 1935; BJÖRNSSON, 1937; GUNN, 1937; AREY, 1939; HAMAN, 1942; VOLLMANN, 1956; MATSUMOTO, 1962). It has been found that 13–15% of all cycles in the adult woman last the "classic" 28 days (AREY, 1939; VOLLMAN, 1940), and that only 6% are of this duration during adolescence (ENGLE, 1934). The length of the cycle changes during the course of development: it decreases up to the 44th year and then rapidly increases again in the premenopause (Fig. 30). The cycle lasts 21–35 days in 90% of adult women (AREY, 1939; MATSUMOTO, 1962). (Fig. 31).

The menstrual interval is prone to variations even in the same woman (Fig. 31). Only 1%

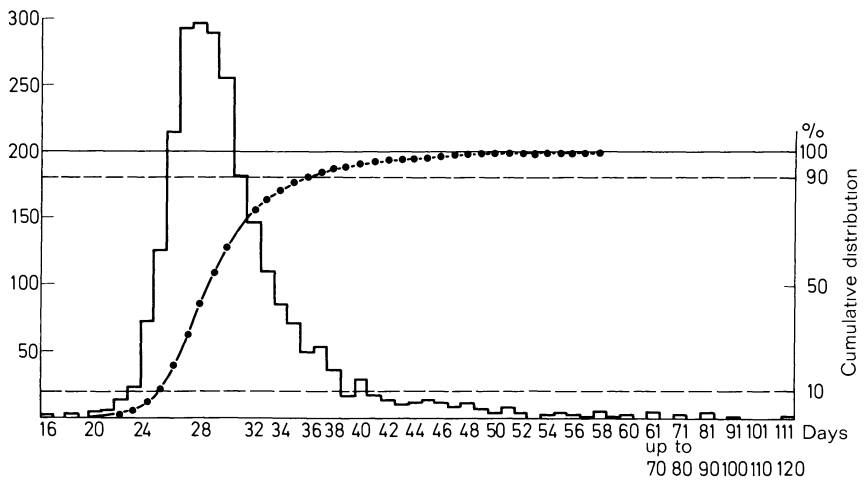


Fig. 31. Frequency and cumulative distribution of length of cycle in 2500 ovulatory cycles in 2500 women aged 20–30 (MATSUMOTO, 1962)

demonstrate a difference of not more than 2 days within a year (LATZ, 1935), whereas 20% show a difference of more than 7 days (LATZ, 1935; GUNN, 1937). FRÄNKEL's statement that irregularity is the only regular feature of the menstrual period (1911) is still true even today. Variations in the duration of the cycle are least common between the ages of 30 and 44 (VOLLMAN, 1956; MATSUMOTO, 1962). They are especially common at the beginning and end of sexual maturity, due to the higher number of anovulatory cycles at these times (Table 58).

More recent investigations (FLUHMANN, 1957; VOLLMAN, In: HARTMAN, 1962; MATSUMOTO, 1962; DÖRING, 1966) have confirmed that the corpus luteal phase lasts about 14 days. This was first determined by KNAUS (1929). The hyperthermal phase lasts 12.65 ± 1.63 days on average (MATSUMOTO, 1962) or 12.7 ± 1.7 days (DÖRING, 1966). VOLLMAN (in: HARTMAN, 1962), however, examined 2081 basal temperature curves and found that the hyperthermal phase lasted 14 ± 2 days in only 42.5% (Fig. 30). On the other hand, the preovulatory phase is much more subject to variation.

The *duration of menstruation* varies between 3 and 7 days in 80–90% of normally menstruating women (VASQUEZ-ROCHA, 1943; MATSUMOTO, 1962). FLUHMAN (1934) arrived at similar figures, finding a mean duration of 4.6 days. The duration of bleeding decreases slightly with increasing age (MATSUMOTO, 1962), although menstruation is prolonged with anovulatory cycles (MATSUMOTO, 1962) (Fig. 31).

Information obtained from women investigated about the *severity* of bleeding is very subjective. The number of tampons used gives only an inexact picture of the severity of bleeding, and 8–10 sanitary pads may very well be an average.

More reliable information on the loss of menstrual blood is gained from examination of the amount of iron in the menstrual blood collected (BARER, 1939) or the hemoglobin content of this blood (RYBO, 1966), or by using Fe 59 or erythrocytes labeled with Cr 51 as tracers (BALDWIN, 1961; GÖLTNER, 1964; PRICE, 1964; MATSUMOTO, 1962). The mean blood loss in the normally menstruating woman is 25–50 ml, but varies between 5–180 ml. Bleeding is usually at its maximum on the 2nd day (RYBO, 1966), and 79% of the blood is lost in the first two days (RYBO, 1966). Blood loss becomes less severe with increasing age (MATSUMOTO, 1962). It is slighter in nullipara than in multipara (RYBO, 1966). The monthly loss of iron is 3–30 mg, mean 12 mg (HYTTEN, 1964). The amount of iron (about 120 mg) saved during a pregnancy is thus rather small in comparison to the iron requirements (500 mg during pregnancy, after HYTTEN, 1964). The monthly loss of iron can, however, result in iron-deficiency anemia in poor nutritional states. Thus for example, about 13% women must be refused as blood donors because of anemia, in contrast to 1% of men (HERVEY, 1952).

Menstrual blood does not clot, because of the proteolytic and fibrinolytic enzymes liberated by the decomposing endometrium (PHILLIPS, 1956; ALBRECHSTEN, 1956; FUHRMANN, 1962; BELLER, 1964, 1968; WEISS, 1968). It does not contain fibrinogen, and most of the other factors are diminished. Concentrations of erythrocytes and thrombocytes are also reduced.

Estrogens prevent ovarian atrophy in adult hypophysectomized animals. Follicular atresia fails to occur. These "estrogen ovaries", however, have no follicles with antrum formation, and the interstitial tissue is reduced. Estrogens increase mitotic activity of granulosa cells in the follicles during the antrum phase, and thus cause follicular growth. They are also thought to increase follicular sensitivity to FSH at the same time, whereas progesterone inhibits follicular growth by a contrary action (DICZFALUSY, 1961). Estrogens promote the growth of the glandular tubules in the breasts and the nipples; progesterone and estrogens combine to produce development of the alveoli (FOLLEY, 1948).

b) Extragenital Actions

General effects of estrogens are: the female figure, fat distribution around the hips, development of the female-shaped pelvis, failure of laryngeal conversion, feminine distribution of pubic hair. High doses cause pigmentation of the nipples, labia minora and linea alba. Estrogens exert a mild anabolic effect on the whole body, particularly on the secondary sexual characteristics. Gestagens, in contrast, have a catabolic action. Sexual steroids influence the autonomic reactive state, presumably via autonomic centers in the posterior hypothalamus. There is a total autonomic switch-over during the course of a biphasic ovarian cycle, at ovulation and at menstruation (Fig. 32). (HOFF, 1944; HESS, 1948); during the follicular phase, the parasympathetic system (trophotropic-endophylactic system) becomes particularly reactive, whereas the sympathetic system (ergotropic-dynamogenic) is predominant during the corpus luteal phase (ARTNER, 1954, 1959, 1960; HAUSER, 1960).

Estrogens accelerate epiphyseal fusion and lead to increased calcium deposition in the

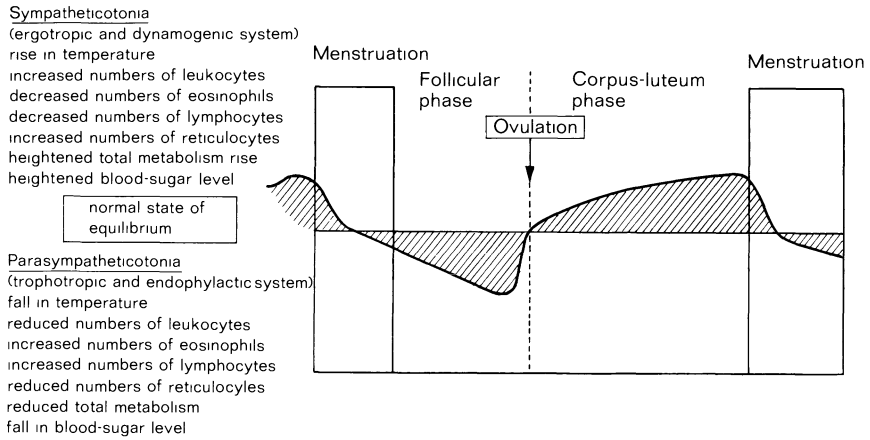


Fig. 32. Changes in the autonomic reaction situation during the biphasic cycle

bones by the stimulation of osteoblastic activity (LANDAU, 1955; GOLDZIEHER, 1956; JOWSEY, 1963; LAURITZEN, 1968). These effects have been observed in cases of gonadal dysgenis treated with estrogens. Gestagens appear to increase calcium mobilization from the bones.

Estrogens influence the composition of the blood by increasing the protein component (DANFORTH, 1946; DÖRING, 1951) and water content (ENGSTRÖM, 1952). The increase in transcortin causes elevated values of the total cortisol although the concentration of free cortisol is unchanged (LAIDLAW, 1962). The concentration of protein-bound iodine also increases (ENGBRING, 1954). Estrogens cause a fall in serum cholesterol levels and in the cholesterol-phospholipid quotient in the blood (KAUFMANN, 1928, 1930; OLIVER, 1953). Oxidative phosphorylation increases in the individual cells under the action of estrogens. ATP formation is doubled, and the uptake of amino acids and phosphates is increased. Protein and RNA synthesis is raised (VILLEE, 1963). Gestagens do not effect oxidative phosphorylation, but more energy for biological use is liberated by activation of adenosine triphosphatase per time unit (SCHREINER, 1965) and amino acid turnover is increased at least in the liver cells. Estrogens cause an increase in extracellular interstitial fluid. Estrogens are assumed to act directly on tissues, causing increased cell and capillary permeability (BUCHHOLZ, 1953) as well as depolymerization of tissue polysaccarides (FRIEDBERG, 1959). 15–20 mg estradiol benzoate causes a mean weight increase of 1 kg after 7–10 days (Fig. 33, LAURITZEN). Gestagens primarily have the opposite effect, since they temporarily increase sodium excretion in the urine. The water-retaining effect of estrogens is especially pronounced when combined with the action of gestagens, as is the case premenstrually (Fig. 34). This secondary synergistic action of estro-

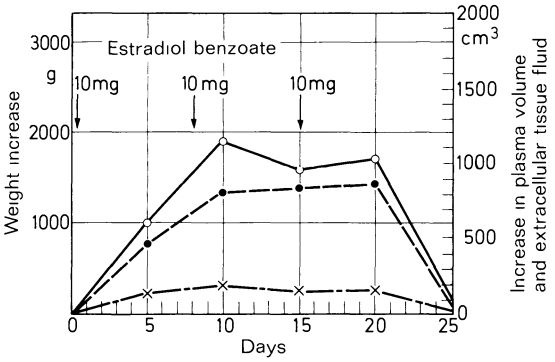


Fig. 33. Increase in weight (○ ——— ○), volume of plasma (× – – – ×) and interstitial tissue fluid (● ——— ●) after administration of 30 mg estradiol benzoate to a castrated woman. (After LAURITZEN, 1961)

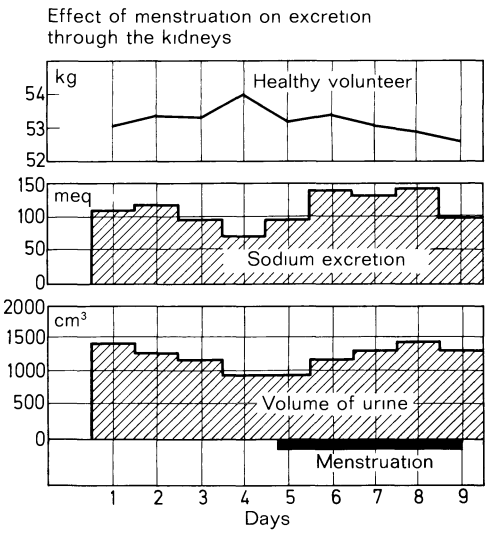


Fig. 34. Effect of menstruation on excretion through the kidneys

gens and gestagens on water retention may be due to the primary natriuretic action of progesterone causing an increase in the secretion of aldosterone and possibly also of desoxycorticosterone (LAIDLAW, 1962). The physiological interstitial water retention present premenstrually is about 700–1500 g (RÖTTGER, 1957) but can be very much more and reach pathologic amounts (up to 6 liters), giving rise clinically to the premenstrual syndrome (p. 616). The hyperemic effect of estrogens in the genital organs, skin, brain and myocardium is probably due to an increase in the amounts of acetylcholine released locally (REYNOLDS, 1931). Estrogens exert a definite hypothermal effect (Fig. 35),

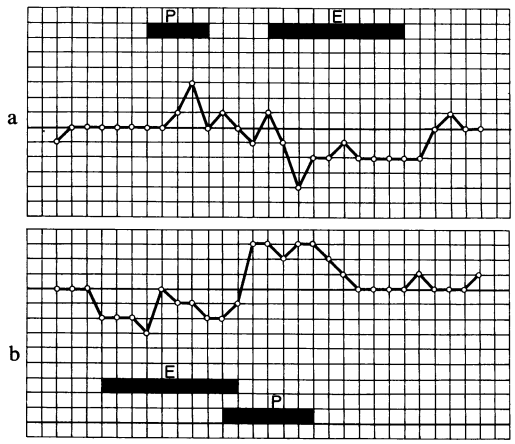


Fig. 35a and b. Effect of progesterone and estrogen on the basal temperature (after ISRAEL, 1950). a) In the castrated woman progesterone causes the basal temperature to rise, while estrogens cause it to fall. b) The same dose of progesterone causes a more pronounced rise in basal temperature after previous administration of estrogens. *P* progesterone, *E* estrogen

whereas gestagens have a thermogenic action causing a rise of 0.4–0.6 °C in temperature during the cycle (BUXTON, 1948; OBER, 1951) (Fig. 35). This is probably due to a central action (ELERT, 1951). The basal temperature curve has a biphasic course in a normal biphasic ovarian cycle (Fig. 69). The action of estrogens on the hypothalamus is dependent on the type and amount of the estrogen, and on the duration and time of onset of the action. Small doses of estradiol and estrone stimulate gonadotropin excretion (SMITH, 1955) (so-called positive feedback). Large doses also produce a temporary increase, probably by stimulating LH release (BROWN, 1947; VORYS, 1965). Short-term estrogen therapy is therefore thought to be successful in a few cases of hypophyseal-diencephalic amenorrhea (KUPPERMAN, 1958). High doses of estrogens for several days reduce gonadotropin excretion to practically nil (WERTH, 1955). The central inhibitory effect of progesterone is significantly less, and 100–400 mg progesterone is required daily to prevent gonadotropin excretion (BUCHHOLZ, 1959, 1964). The central antigonadotropic action of estrogens has gained practical importance in the field of ovulostatics.

Gonadotropin production, secretion and excretion may be increased after discontinuation of long-term estrogen treatment (so-called rebound phenomenon) (HELLER, 1944; HOHLWEG, 1952). This is a secondary effect of inhibition therapy with estrogens, which used to be commonly applied in cases of anovulatory cycles.

Progestagens and gestagens have different activity spectra (EDGREN, 1967) (Table 14). In addition, individual progestagens differ considerably in their biological activity (Fig. 36, SZONTAGH). It is therefore impossible simply to compare their progestative action, and the results obtained depend on the test used. Single progestative properties can be strengthened or diminished by changing the side chains of the steroid nucleus. Thus, for example, the central inhibitory action is particularly pronounced for derivatives of 19-nortestoids and 17α-hydroxy-progesterone, and is one of the three factors essential for the contraceptive action of the "pill" (p. 568). All progestagens have the ability to convert proliferative endometrium (due to estrogens) into secretory endometrium. With the exception of retroprogesterone, progestagens such as progesterone have a definite thermogenic action.

Table 14. Properties of progestins. (After KISTNER, 1964)

Test	Progesterone	17α-hydroxyprogesterone capronate	Medroxy progesterone	Norethisterone	Norethynodrel
Clauberg	+	+	+ + + + +	+	+ ($\frac{1}{4}$)
McGinty	+	+	+ (× 25)	−	−
Deciduoma	+	+	+	−	−
+ Pregnancy	+	+	+ +	−	−
↑ Seminal vesicle	−	−	+	+	+
♀–♂	−	−	+	+	+
↓ Gonadotropin	±	+	−	+ +	+ + +

Most progestagens given orally are many times more potent than progesterone. Their activity is measured in the endometrium and is connected with the stability of the molecule. Progesterone and the ground substances of progestagens, 17α-hydroxy-progesterone and 19-nortestosterone are all typical intermediate products of steroid biosynthesis and are extremely fragile molecules. The stability of these molecules can be increased by changing the molecular structure, by methylation or chlorination at C 6, and by esterification or ethylation at C 17. From these and other processes it has been found that a ketone group at C 3 is not essential for various progestive effects.

Because of these structural changes, progestagens are broken down differently from progesterone. Progestagens are not excreted as pregnanediol. As expected, only a small amount of 19-nortestoids is metabolized into estrogens. They therefore also exert a slight estrogenic effect in addition to their progestative and weak androgenic actions. Regulation of the cycle is therefore more effective than with derivatives of hydroxy-progesterone, which possess no estrogenic action. Breakthrough bleeding and amenorrhea due to atrophy of the endometrium occur less commonly. In contrast, the anti-estrogenic effect of hydroxy-progesterone derivatives is marked. The anti-estrogenic action due to inhibition of the production of endogenous estrogens is, however, not compensated (p. 575) by the amounts of estrogens used in ovulostatics (Table 37).

Progestagens with the $CO\text{-}CH_3$ group at C 17 in the β position and a ketone group at C 20 (derivatives of 17α-hydroxy progesterone) act catabolically, like progesterone, and temporarily increase the excretion of sodium chloride. Progestagens with the side chain at C 17 in the α position (derivates of 19-nortestosterone) have a salt-retaining effect, as do estrogens (LANDAU, 1958).

6. Methods of Estimation and Standards

a) Estrogens

Estrogenic substances can be demonstrated by biological, biochemical, physical and chemical means. The extreme sensitivity of vaginal epithelium to estrogen hormones is put to practical use in the *Allen-Doisy test* (1923) for biological semi-quantitative testing of estrogens. Castrated adult female mice or rats are used as experimental animals. A mouse unit (MU) or rat unit (RU) is the minimum amount of estrogens which produces the estrus phase in the vaginal epithelium in at least 50% of the experimental animals. In 1932, the MU and RU were replaced by the international unit (IU), whose potency corresponds to that of 0.1 μg estrone. It is that amount of test substance which when injected subcutaneously just results in a positive estrus phase in 50% of the castrated female mice. With the introduction of estrogen esters into the therapeutic field, a second standard (the international benzoate unit, IBU) was created for estradiol-3-monobenzoate in 1935. This unit corresponds to the estrogenic activity of 0.1 μg estradiol benzoate.

The following equation is valid:

$$1\ \text{MU} \cong 0.2\ \text{RU} \cong 1\ \text{IU} \cong 0.1\ \mu\text{g estrone.}$$

These equations are only valid for the Allen-Doisy vaginal keratization test, but the values can deviate by several hundred percent. The international unit was abolished as a comparative mass in 1950, since the most important estrogens

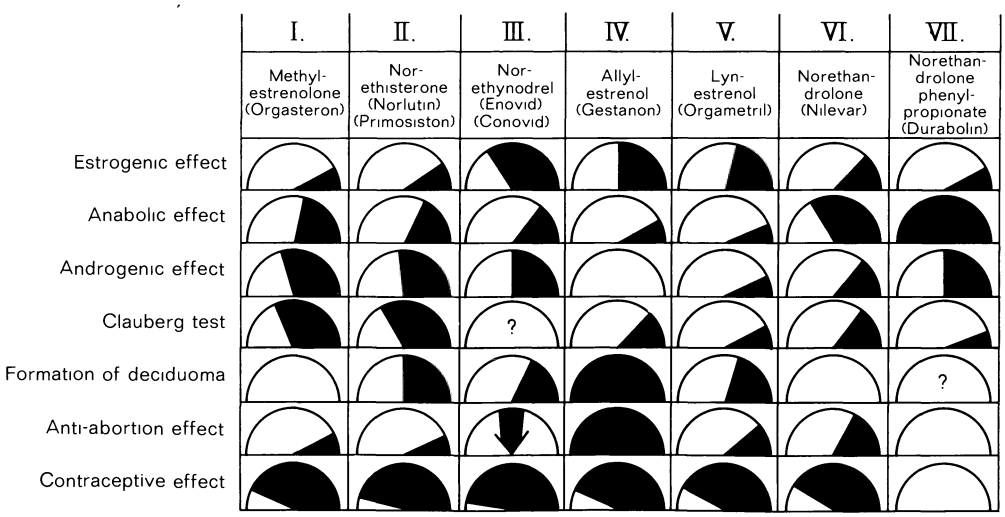


Fig. 36. Spectra of effects of various progestins (SZONTAGH, 1967)

were produced in the purified form and the weight unit could be given.

Instead of standardization in units, information is presented as the number of milligrams required to produce estrus in 50% of the experimental animals. A comparison of the approximate activity of the different estrogens measured in the vaginal epithelium or by uterine growth in the castrated adult mouse following s.c. administration gives to the following picture:

1 mg 17β-estradiol	120000 IU or MU
1 mg estrone	10000 IU or MU (by definition)
1 mg estriol	1500 IU or MU

Thus the potency of estradiol to estrone to estriol is roughly in the ratio 100 : 10 : 1.

1 mg estradiol benzoate	60000 IU or MU
1 mg di-ethylstilbestrol	50000 IU or MU

In comparison to this, the potency of 1 mg conjugated estrogen is equivalent to about 3500 IU of MU given orally.

Since 1955, reliable, quantitative chemical methods have become available for the measurement of estrogen excretion in the urine (BROWN, 1955, 1957; ITTRICH, 1958, 1960). They are based on colorimetric estimation of isolated urinary estrogens with the aid of the Kober reaction. This reaction is based on the fact that estrogens produce a red coloration under certain conditions with a mixture of phenols and sulfuric acid. In our experience, estimation of the excretion of the three classic estrogens for practical purposes is best by BROWN's modified method using 24-hour urine (BROWN, 1957). This test consists of: acidic hydrolysis of the conjugated steroids, several stages of extraction, purification of the ether extracts and separation of the phenol fraction, methylation, chromatographic separation of the different estrogen fractions in aluminium oxide, and quantitative estimation of these fractions as 3-methyl ether by colorimetric means using the Kober reaction. The sensitivity limits lie between 0.6 and 0.8 μg/24 h.

Quantitative demonstration of minute amounts of estrogens such as are found for example in the plasma can be performed by means of ultra-violet spectrophotometry, gas chromatography or radioisotope methods (SVENDSEN, 1960). These methods are, however, available only in a few special laboratories at present.

b) Gestagens

The endometrium of a rodent pretreated with estrogens is the classic substrate for testing the progestative action of a substance. The *Allen-Corner test* (1929) is performed in ovariectomized adult rabbits, and the *Clauberg test* (1930) in juvenile female rabbits.

The rabbit unit of ALLEN and CORNER and of CLAUBERG corresponds to the smallest amount of a test substance which causes complete secretory transformation in the proliferative endometrium when injected in 5 equal daily doses into the experimental animal.

A Clauberg unit corresponds to the progestative action of 0.6 mg progesterone, and the Allen-Corner rabbit unit to that of 1.2 mg progesterone.

The following approximate equation is valid:

1 Allen-Corner rabbit unit
≅ 2 Clauberg rabbit units
≅ 1 international unit
= 1 mg progesterone.

The Allen-Corner and Clauberg tests are not sensitive enough for clinical purposes. They have therefore been modified repeatedly (Table 15) by increasing the sensitivity by local intrauterine application of the test substance (McPhail test, McGinty test).

Table 15. Biological methods for estimation of progestational activity

1929	Corner-Allen test	Adult female rabbits castrated 18 h after mating
1930	Clauberg test	Young female rabbits pretreated with estrogen
1934	McPhail test	Modification of the Clauberg test
1939	McGinty test	Modification of the McPhail test; the test substance is given by the intra-uterine route
1947	Hooker-Forbes test	Ovariectomized mice, to which the test substance is administered by direct injection into the lumen of a ligated uterine segment (hypertrophy of stroma-cell nuclei in the endometrium)

The *Hooker-Forbes test* is the most sensitive biological test for gestagens (1947). 0.0002 μg progesterone is sufficient to produce hypertrophy of the nuclei of the stroma cells of the endometrium of the mouse. The Hooker-Forbes microunit corresponds to the progestative action of 0.0002 μg progesterone. This test is, however, not very specific.

The progestative action of a substance may vary considerably within one species. Reservation is therefore indicated when the results obtained from experiments with animals are

extrapolated to the woman. For example, 17α-hydroxy-progesterone is about 60 times more active than progesterone in the mouse, whereas it has no progestative effect in the human and rabbit (SALHANICK, 1957). Progesterone and 20α(β)-hydroxyprogesterone can be demonstrated reliably during the cycle by gas chromatography in the blood, urine, and tissue. This method is not suitable for routine work in the clinical laboratory, however, and is used for research into special scientific problems.

The methods used for practical clinical purposes rely on the pregnanediol excretion in the 24-hour urine for assessment of the production of endogenous progesterone. Since pregnanediol is derived almost entirely from progesterone, its concentration in the urine reflects the endogenous progesterone production. There are numerous methods of estimation, but the chromatographic method of KLOPPER, MITCHIE and BROWN (1955) has proved the most satisfactory for practical clinical requirements. It is specific and sensitive. The limits of sensitivity are 0.5–1.0 mg/liter. The method consists of: acidic hydrolysis, extraction and purification of the extracts, adsorption chromatography with aluminium oxide, acetylation, purification by oxidation with potassium permanganate, repeated adsorption chromatography and colorimetric estimation of pregnanediol acetate.

Since most pregnanediol is derived from ovarian progesterone, its measurement serves to assess the function of the corpus luteum. It must be remembered, however, that even in normal conditions quite a considerable amount of pregnanediol arises from the adrenals.

Pregnanediol can also be quantitatively estimated by thin-layer (SULIMOVICI, 1965) and gas chromatography (TURNER, 1963).

7. Modes of Administration and General Principles for Adjusting the Doses of Ovarian Hormones

The actions and side effects of ovarian hormones must be known for successful therapeutic results as precisely as possible. Modes of administration, dosage, time and duration of treatment all affect the result substantially. Together with the nature of the compound, they determine the time interval before onset of action, potency, duration of action and possible side effects. Examination of one compound therefore necessitates a considerable time and a necessary number of patients in the same initial condition. It is therefore also essential, even in a large clinic, to use a selection of the numerous drugs available, in order to obtain the necessary experience. Limitation to a few preparations does not necessarily qualify the remaining commercial compounds, since numerous excellent hormone compounds are now on the market.

The following conditions are essential in hormone treatment:

1. the treatment should take up as little as possible of the patient's and the doctor's time;
2. an exact dosage regime should be possible, and it should be possible to estimate the effect in advance;
3. treatment should be as economical as possible.

The results obtained with laboratory animals are only of limited value, and can be extrapolated to the woman only with serious reservations. Only results obtained from clinical trials in the woman can be appealed to in decisions on clinical treatment. The following factors must be considered:

a) the dose required to produce a certain clinical effect (potency);
b) time lapse before onset of action;
c) duration of action;
d) further specifically sexual and general side effects.

The activity threshold must be crossed before a reaction can occur. The threshold value depends on the receptor organ i.e. on the sensitivity of the receptor. The concentration at the site of action, the time lapse before onset of action, and duration of action of a given dose are dependent on the speed of absorption, conversion, and breakdown in the organism and the speed of excretion. They vary according to the mode of administration and the compound used. A series of tests have been developed, based on the numerous receptor organs and the activity qualities of an ovarian hormone. Every fact given about the activity qualities of a sexual steroid should be related to an appropriate test. As they are produced in purified forms steroids are dosed in milligrams. In earlier literature biological units were used since purification of the hormone concerned was not possible at that time. In the evaluation of results obtained from these tests in women, it must be remembered that these results are not statistically significant because it is simply not possible to test a large enough group of suitable women. These results are empirical, and increase in value as the number of women in comparable initial conditions subjected to the treatment under consideration rises.

We differentiate three principal forms of hormone treatment: stimulation, substitution and inhibition.

In cases where hypothalamo-hypophyseal function is absent or insufficient and the ovaries are intact, ovarian function can be successfully reestablished by *stimulation* with hypophyseal gonadotropins. If there is peripheral ovarian failure, the hormonal deficit can only be compensated for by appropriate *substitution* of the absent ovarian hormones.

High doses of ovarian hormones cause temporary ovarian failure by *inhibition* of gonadotropic function of the anterior pituitary. After discontinuation of hormone administration, gonadotropin secretion is rapidly resumed and the amount released may even be elevated (so-called rebound phenomenon). Previously inadequate ovarian function can be normalized in such cases.

Forms of Administration

Ovarian hormones are now available in pure crystallized form. They are either produced synthetically or gained from biological material.

They can be administered orally, parenterally or topically. Depending on the form of application, the same dose gives different absorption times and thus different clinical results (Fig. 37).

Oral administration is simple and economical and facilitates regular hormone intake over a definite time. The oral route is therefore the route of choice. Natural ovarian hormones are absorbed in the small intestine and are subsequently rapidly inactivated in the liver. Because of this, synthetic compounds with corresponding actions are used instead. These substances belong to different groups: 17α-alkylized steroids, such as 17α-ethinyl-estradiol (INHOFFEN, 1938) which is approximately 100 times more effective than estradiol, and synthetic progestagens (19-nortestoids, derivatives of 17α-acetoxyprogesterone) (Figs. 45, 46); when given orally stilbenes (p. 523), non-steroid substances, stilbestrol and chlorotrianisene (Tace), which are stored in adipose tissue and are slowly released, give rise to a depot action. Methallenestril (Vallestril) is also orally effective. Finally, conjugated estrogens must be mentioned. These are a mixture of esters of sulfuric and glucuronic acids of natural estrogens, particularly of estrone and its metabolites. They are fully effective orally since they are water-soluble. They have only a slight effect on the endometrium. Withdrawal and breakthrough bleeding seldom occurs and they are therefore termed as "soft" estrogens in contrast to the "hard" synthetic estrogens. Their effect on the vaginal epithelium is thought to be almost twice as great as that of nonesterized estrogens (GRANT, 1950).

Buccal (sublingual) administration of ovarian steroids to avoid the portal circulation of the liver is little used. Natural estrogens exert a definite although slight effect by this route. It is seldom necessary to give ovarian steroids parenterally. As lipoids they are easily soluble in oil, and can be given intramuscularly in the natural form or as the ester disolved in oil. Natural estrogens are rapidly absorbed; a peak is reached in the plasma on the first day, and a fall with loss of activity occurs after 2–3 days (Fig. 38). Thus, if a longer duration of action is required, administration should be repeated every second day. Esterification with a fatty acid causes a depot action resulting in retarded absorption (Fig. 38). Decomposition and excretion may also be delayed. The duration of action is dependent on the molecular weight of the ester and the dose (Table 16). An analogous depot action is achieved by intramuscular administration of microcrystals suspended in aqueous solution. The rate of absorption is

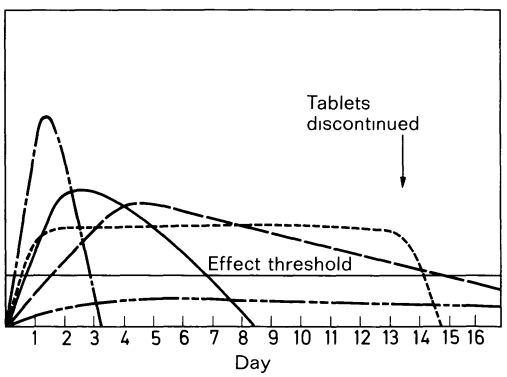


Fig. 37. Schematic comparison of the effectivity of various drug forms (UFER, 1966). ——— i.m. injection, – – – – – prolonged-action injection, ---—---—--- hormone implant, ---------- tablets, — - — - — i.v. injection

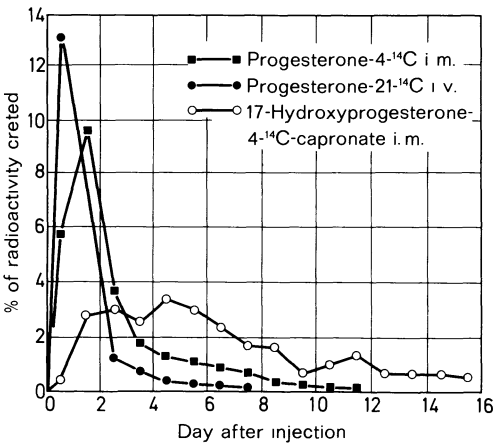


Fig. 38. Different speeds of excretion with radiolabeled gestagens. (After PLOTZ, 1960)

determined by the length of the edge of the crystals. It is, however, often dependent on tissue reaction and circulation variations which impair the desired clinical effect, so they are now seldom used.

Table 16. Examples of the varying duration of effect and saturation dose of ester compounds of estradiol. (After UFER, 1966)

Steroid	Single dose (mg)	Duration effect (days)	Saturation dose (mg)
Estradiol	10	2	40–60
Estradiol benzoate	5	6	25–35
Estradiol valerianate	10	10–14	20
Estradiol undecylate	10	40–60	–

The polymer of estradiol or estriol and phosphoric acid has an excellent depot action when given intramuscularly (polyestriol phosphate or polyestradiol phosphate) (Table 16). Phosphoric acid combines with the estrogen molecule at C 3 and C 17 to form a macromolecule. The compound is stored in the liver and spleen where the estrogen is steadily released by the splitting off of the phosphate portion due to the action of alkaline phosphatase.

Conjugated estrogens and polyestriol and estradiol phosphate can also be given intravenously in an aqueous solution. *Intravenous* administration of ovarian hormones offers no advantages, however, and therefore has no practical significance.

Implantation of crystal pellets into the tissue is now practically obsolete because of the danger of severe uncontrollable hemorrhage.

Ovarian hormones are used *topically* in the form of vaginal ovula and tablets, pessaries, creams and lotions. Rectal application to avoid the liver has gained no practical importance. The same is true of percutaneous administration, where addition of alcohol or menthol improves absorption.

a) Estrogens

The therapeutic importance of estrogens is connected with their specific action on the growth of the female genitalia and mammae, their controlling action on gonadotropic secretion and their influence on specific metabolic processes.

Estrogen treatment has proved satisfactory for the following indications:

1. promotion of development of primary and secondary sexual characteristics (gonadal dysgenesia, ovarian hypoplasia);
2. dysfunctional bleeding, including functional amenorrhea;
3. endometriosis, when used together with progestagens;
4. for improvement of the cervix factor in sterility;
5. suppression of laction;
6. inhibition of ovulation in functional dysmenorrhea and for contraception;
7. prevention of climacteric syndrome (vasomotor and psychological disorders, osteoporosis, atherosclerosis);
8. inhibition of excessive growth during female adolescence.

Undesirable *side effects* which may develop are gastric complaints with nausea when used orally, especially with stilbenes; cyclic disturbances through displacement of ovulation; painful swelling of the breasts, possibly mastopathia cystica; hemorrhage after implantation of crystal pellets or with too high doses during the post menopause; edema due to sodium retention.

There is no evidence of any causal connection between estrogen therapy and carcinoma of the uterus and the breast. A series of methods have been developed to test the *potency and duration of action* of estrogenic substances in the woman. These vary with the receptor organ, and it is essential to give the receptor organ when stating the action of an estrogen.

The vaginal epithelium is the most sensitive test organ reacting to estrogens. The test uses atrophic epithelium from an elderly or a castrated woman, from which hormonal cytological smears are made (p. 534). From these tests it has been deduced that a daily dose of less than 40 µg but more than 20 µg estradiol is necessary to build up the atrophic epithelium completely after 10 days (OBER, 1957). Generally, 10 mg of an estradiol ester given intramuscularly is sufficient (Fig. 39). Vaginal epithelium reacts slowly, and 3–6 days are required for a definite build-up to occur, and 5–10 days for full proliferation. Actual hormonal states and variations can therefore not be detected by this method. Vaginal cytology is particularly suitable for assessing the duration of action of an estrogen (WIED, 1954) (Fig. 39).

The dose of estrogens required to build up the resting endometrium to the full proliferative phase (so-called build-up dose) in the woman is of practical therapeutic interest (Table 16). KAUFMANN determined this dose in a trial with ovariectomized women with intact uterus or in woman with functional amenorrhea and with uterus of normal size (FERIN, 1952). According to KAUFMANN (1932), 25–30 mg estradiol benzoate given in divided doses over 3–4 weeks is required to convert

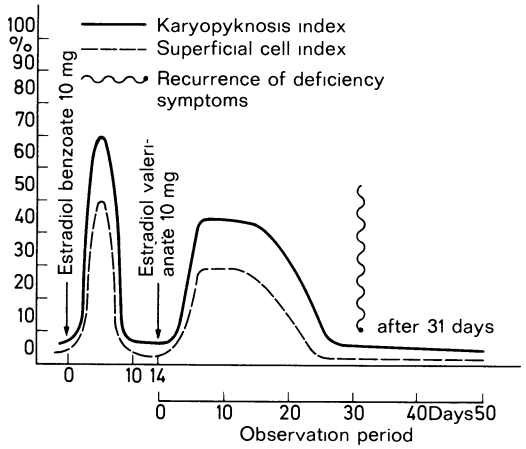


Fig. 39. Effect of 10 mg estradiol benzoate and 10 mg estradiol valerianate on atrophic vaginal epithelium in a 55-year-old woman. (After WIED, 1954)

the resting endometrium to its full proliferative phase. Lower doses are adequate if the estrogens are very potent, e.g. 3 mg over 4 weeks when estradiol crystals are implanted (GIESSEN, 1943). This corresponds roughly to the amount of estrogens produced per cycle (p. 527). 2 mg ethinylestradiol given orally over 3 weeks is sufficient to produce full proliferation. Too high doses of estrogens in the first half of the cycle prevent secretory transformation by causing stromal edema. BISHOP (1950) tested the potency of different oral estrogens by means of withdrawal bleeding in women with functional amenorrhea. If the activity of stilbestrol is set at 100, the potencies for other estrogens are:

Table 17. Efficacy of various estrogens in inhibiting ovulation. (After MARTINEZ-MANAUTOU, 1966)

Substance	Dose	Cases	Cycles	Ovulatory cycles
Ethinyl estradiol	20 µg	10	20	2
Ethinyl estradiol	50 µg	20	44	1[a]
Ethinyl estradiol-3-methyl-ether	20 µg	10	20	2
Ethinyl estradiol-3-methyl-ether	80 µg	18	60	1
Estradiol	1 mg	4	11	5
Estradiol	2 mg	10	18	7
Estradiol	5 mg	10	24	3
Estriol	5 mg	5	7	6
Premarin	1.25 mg	10	18	12
Premarin	3.75 mg	15	17	1
Stilbestrol	5 mg	6	12	1
Total		118	251	41

[a] Secretory endometrium in 12th cycle in one patient.

Stilbestrol	100	Ethinyl estradiol	2562
Dienestrol	26	Stilbestrol sulfate	44
Hexestrol	5	Estrone	5

The duration of administration before onset of withdrawal bleeding also permits comparison of the duration of action of different estrogen preparations (FERIN, 1952). Withdrawal bleeding occurs 4–6 days after discontinuation of orally administered estrogens. The bleeding threshold is exceeded by a daily dose of 50 µg ethinyl estradiol. Potency and duration of action of an estrogen preparation can be tested using the disappearance or reappearance of the post menopausal syndrome as an indicator. To exclude possible psychological influences, tests of this kind should always be performed as double blind trials against placebo.

The following duarations of action have been obtained with a single administration (WIED, 1954; LAURITZEN, 1968):

Table 18. Methods for experimental and clinical testing of gestagens

Animal experimentation	Clinical trials
	Kaufmann trial
	Withdrawal bleeding in secondary amenorrhea
	Manipulation of menstruation
Clauberg test Deciduoma test	Testing on the vaginal epithelium
Testing for local efficacy (MCGINTY, HOOKER-FORBES)	
Anti-oxytocin test on the pregnant rabbit (CSAPO)	Anti-oxytocin test in saline-induced abortion (BENGTSON)
Anticycle test	Quantitative estimation of gonadotropins in urine
Anti-ICSH-Test (reduction of weight of testes)	Determination of the dose inhibiting ovulation (PINCUS)
Androgenic effect on cockscomb and test on seminal vesicle	–
Fetal virilization	–
Estrogenic effectivity (Allen-Doisy test)	Testing on vaginal epithelium Estimation of estrogen metabolites
Anabolic efficacy (levator-ani test)	Metabolic equilibrium
Anti-ACTH effect (determination of weight of adrenal cortex)	Estimation of corticoid metabolites

10 mg estradiol valerianate ~ 1 month
50 mg polyestradiol phosphate ~ 1 month
50 mg polyestriol phosphate ~ 1 month
80 mg polyestriol phosphate ~ 2 months
25 mg estradiol undecylenate ~ $2\frac{1}{2}$ months
50 mg estradiol undecylenate ~ 3 months

The central antigonadotropic action of estrogenic substances is reflected in their efficacy as contraceptives. Ethinyl estradiol (EE) and its 3-methyl ester (mestranol = EEME) are orally much more effective than stilbestrol or conjugated estrogens (Table 17). Doses of as little as 0.05 mg EE or 0.08 mg mestranol daily have proved to be effective inhibitors of ovulation.

b) Progestins

The therapeutic use of progestins is based both on the action of gestagens and various progestagens in preserving pregnancy, and on the differentiating effect exerted on end structures made to proliferate under the influence of estrogens.

They are therefore indicated in the following circumstances (ROLAND, 1965; KISTNER, 1967):

1. threatened abortion;
2. dysfunctional bleeding and amenorrhea (usually combined with estrogens);
3. endometriosis;
4. for contraception and in combination with estrogens for functional dysmenorrhea;
5. inoperable carcinoma of the body of the uterus and the breast.

Even high doses of gestagens produce no undesirable side effects, but progestagens may have side effects because of the activity of the androgenic component. Thus, for example, 19-nortestoids are contraindicated during pregnancy because of the possible virilizing effect on the female fetus (p. 574).

There is a series of methods for testing the very variable spectrum of action of progestins (progestive, androgenic, estrogenicaction) (UFER), and the duration of progestative action and potency (Table 18).

If the ovaries of a sexually mature woman are removed premenstrually, withdrawal bleeding occurs on average 36 hours later. The time

Table 19. Relative efficacy of various progestins, assessed by the amounts needed to produce withdrawal bleeding (in patients with adequate endogenous estrogen or receiving appropriate amounts of exogenous estrogens). (After AYDAR, 1961)

Substance	Dose (mg/d for 5 days)	Total dose (mg)	Time-lapse before onset of withdrawal bleeding (h)
Progesterone	90 –100	450 –500	24–72
Medroxyprogesterone acetate	2.5– 5	12.5– 25	24–72
Ethisterone	20 – 30	100 –150	24–72
Dimethisterone	10 – 15	50 – 75	24–72
Norethynodrel	2.5– 5	12.5– 25	24–72
Allylestrenol	10 – 15	50 – 75	24–72

Table 20. Doses of different gestagens needed to obtain various effects. (After NEVINNY-STICKEL, 1964)

Compound	Inhibition of ovulation	Minimum doses (mg) necessary for:			
		Transformation of endometrium	Manipulation of menstruation	Arrest of functional bleeding	Withdrawal bleeding in amenorrhea
6-chloro-6-dehydro-17α-acetoxyprogesterone	1–3	20	4[a]	4[a]	2
1,2α-methylen-6-chloro-dehydro-17α-acetoxyprogesterone	2–3	20	3[a]	3[a]	2
9α-fluoro-11-hydroxy-16-methylen-17α-acetoxyprogesterone	1–4	30	20[a]	4[a]	2
17α-ethinyl-19-nortestosterone acetate	4[a]	40	10	8	4
17α-ethinyl estrenol	5	150	10	–	–
17α-hydroxy-19-norprogesteron-17-acetate	10[a]	100	25[a]	>20	20
17α-allyl estrenol	25	250	30[a]	20	40

[a] When estrogens are added.

interval between progestin administration and the development of withdrawal bleeding in a woman with functional amenorrhea but with a uterus of normal size is another test of the duration of action of a progestin, if the uterus is previously prepared with estrogens.

The potency can be deduced from the dose progestin needed to produce withdrawal bleeding (Table 19).

Displacement of menstruation in a normally menstruating woman is probably the most usual method for estimation of the potency of a progestin (GREENBLATT, 1958; SWYER, 1962) (Table 20).

The *transformation dose* is of therapeutic importance. This is the amount of a progestin necessary to transform the proliferative endometrium into the full secretory phase (Table 21). Awareness of the broad spectrum of activity of progestagens is essential since a compound is usually chosen for a single property while its other potential effects are unwanted.

Table 21. Transformation doses of various progestins (estrogens to day 20, progestin from day 15 onward). (Modification after UFER, 1966)

		Transformation dose
	Free progesterone i.m. 20 mg/day	200 mg
Estrogen	↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	
	17α-hydroxyprogesterone capronate 2 × 125 mg i.m.	250 mg
Estrogen	↓ ↓	
	Medroxyprogesterone acetate 50 mg i.m.	50 mg
Estrogen	↓	
	17α-ethinyl nortesterosterone acetate 2 × 5 mg/day p.o. for 6 days	60 mg
Estrogen	0 0 0 0 0 0 0 0 0 0 0 0	

D. Regulation of Ovarian Function

1. Anatomical and Functional Structure of the Hypothalamo-Hypophyseal System

Vegetative and generative functions of the ovaries are regulated centrally. Reception, integration and modulation of proprioceptive and exteroceptive stimuli influencing ovarian function take place in the nuclear areas of the hypothalamus, where they are converted into neural and neurohumoral impulses, the latter being transmitted to the adenohypophysis via neurovascular routes. The anatomical structure is presented and discussed in another section (Chap. II, p. 27) and it will only be mentioned here when necessary to the explanation of central ovarian disorders.

Our knowledge of the functional segmentation of the hypothalamus and its influence on the pituitary and ovaries is obtained mainly from experiments on rat and rabbit, and also on guinea pig, cat and monkey. Results have been gained from section of the pituitary stalk, pituitary transplantations, selective stimulation or destruction of hypothalamic nuclear areas and their associated structures and from extracts of the eminentia mediana. Clinical features arising from tumors and traumatic lesions in the region of the hypothalamus in man are consistent, to a great extent, with the knowledge obtained from animal experiments and permit this to be referred, with caution, to the human.

The large-celled supraoptic and paraventricular nuclei are found in the anterior region of the hypothalamus. The ganglia of these nuclei produce the neurohormones vasopressin (adiuretin) and oxytocin. Their axons form the part of the hypophyseal tract consisting of thick fibers and ending in the infundibular process. The center responsible for the cyclic secretion of gonadotropins (secondary or cyclic inhibitory center/system) also lies immediately under the paraventricular nucleus in the rostral portion of the hypothalamus. In both sexes, the cyclic secretion of gonadotropins appears to be inborn. Castration of newly born male rats results in cyclic secretion. Administration of testosterone to female rats not more than 5 days old causes the loss of this ability and produces continuous estrus (BARRACLOUGH, 1954, 1955, 1966). The same phenomenon is also caused by the transplantation of ovaries to castrated male rats. The already "masculinized" inhibitory center for gonadotropins in these animals lacks the ability to function rhythmically. Polycystic ovaries are also formed in such cases, and they correspond histologically to the ovaries in Stein-Leventhal syndrome in the human. In addition to testosterone, HCG, estrogens and corticosteroids also have the same effect. Such influences must act during intrauterine life on the early development of these centers in man, and it is not known to what extent these influences are involved in the etiology of the Stein-Leventhal syndrome.

This rostral nuclear area is an inhibitory center, since its destruction also leads to continual FSH secretion and to loss of rhythmic LH release. It is assumed that the inhibitory action of estrogens and androgens on FSH

secretion is exerted by way of this nuclear region (FLERKO and SZENTAGOTHAI, 1957) since, for example, transplantation of the ovary to this region in the castrated rat has an effect analogous to that of substitution with estrogens. On the basis of animal experiments, it is assumed that the sensitivity of this center to sexual steroids is much greater before menarche than during sexual maturity. It is known that the pars distalis of the anterior pituitary already contains gonadotropins a few years before menarche (JOHNSON, 1959), and that prepuberal ovaries are sensitive to gonadotropins (PASCHKIS, 1955), but gonadotropins are not released at this stage. This suggests that the onset of cyclic ovarian function at the time of puberty is due to a decrease of the sensitivity of this inhibitory center to sexual steroids. Indeed, bilateral lesions in the anterior hypothalamus in the rat result in premature puberty. Similar observations have been made in boys with hypothalamic tumors, even though these tumors were situated in the posterior hypothalamic region. It is possible in these cases that there is a lesion of the fibers running from the amygdaloid nucleus to the mamillary body, since in rats precocious puberty can also be produced by bilateral destruction of the amygdaloid nucleus in the temporal lobes.

The limbic system (cingulate gyri, hippocampus, amygdaloid nucleus, mamillary body), which is connected with the hypothalamus by very well developed fiber systems, appears to modulate the integrating ability of the hypothalamus, producing a predominantly inhibitory effect (AKERT, 1964). It is now assumed that hypothalamus and hypothalamo-hypophyseal system receive the most important impulses from the limbic system. It represents centers of the endocrine and autonomic regulation systems directly above the hypothalamus. It receives its afferent signals via the reticular system, i.e. information from the surroundings and interior of the body are transmitted to the limbic system via the reticular formation (visceral brain). Increased inhibitory action of the limbic system on the hypothalamic "sexual center" causes loss of libido and impotence, as in psychomotor epilepsy. Conversely there is no motivated sexual behavior in animals with bilateral lesions of the amygdaloid nucleus. External stimuli lead to pointless motor activity and hypersexuality develops. Psychological and autonomic influences also act on the hypothalamus via the limbic system.

The rostral hypothalamic nuclear region acts via the basal tuberal nuclei (ventro- and dorsomedial nuclei, infundibular nucleus, arcuate nucleus) to inhibit gonadotropin release. These nuclei form the primary or tonic system or center. The axons of these nuclei form the tuberohypophyseal tract, which corresponds to the part of the hypophyseal tract consisting of fine fibers. It contains no Gomori-positive granules. These nerve fibers terminate in the region of the eminentia mediana, which forms the most anterior part of the tuber cinereum and the cranial portion of the neurohypophysis. This area shows a dense primary capillary plexus derived from the hypophyseal portal system (POPA, 1930).

2. Releasing (RH) and Inhibitor (IH) Hormones of the Hypothalamus

Impulses from the tuberal nuclear region are transmitted to the adenohypophysis by means of neurosecretions from the hypothalamic nerve endings in the eminentia mediana. These are extremely active substances which are effective *in vivo* and *in vitro* in doses of a few nanograms/gram pituitary tissue. A dose of one μg of the hormone releasing LH induces ovulation in sexually mature female rats (ARIMURA, 1967). These neurohormones are described as transmitter substances or hypophysiotropins and are classified as releasing (RF) and inhibitor factor (IF) or releasing hormones (RH) and inhibitor hormones (IH) according to the type of action. In addition to oxytocin and vasopressin, there are seven other known, well defined neurohormones in the hypothalamus. Five of these cause liberation of anterior pituitary hormones (RH), and two inhibit their release (IH).

It has been known for some years that extracts of animal hypothalamus demonstrate FRH and LRH activity (HARRIS, 1961, 1964; GUILLEMIN, 1955, 1964; MCCANN, 1960, 1962). In 1967 it was also possible to show these activities in extracts of human hypothalamus (SCHALLY, 1967). The highest concentration of LRH was found in the eminentia mediana, but this amount is still so small that only 6 mg LRH would be obtained from the eminentia mediana of 6000 sheep (JUTISZ, 1966). This is also true of FRH. MATSUO (1971) was the first to isolate LRF in a practically pure form and to determine its chemical structure. LRH is a decapeptide with the following sequence:

Pyrogly–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH_2.

Interestingly enough, this peptide releases not only LH, but also FSH. As different basic peptides (e.g. arginine vasopressin) have a releasing effect on FSH, it has been suggested that the FRH effect of this decapeptide is only

a side effect due to basic groups. There are observations of two principles in the course of isolation.

Methods of investigating FRH activity *in vivo* are based on depletion of the FSH content in the pituitary of male rats (DAVID, 1965). *In vitro*, the liberation of FSH from pituitary cultures is measured (MITTLER, 1964). *In-vitro* experiments have shown that FRH and LRH act directly on pituitary tissue (SCHALLY, 1964). From experiments in animals it can be concluded that RH and IH stored in the eminentia mediana are transmitted to the primary capillary plexus of the hypophyseal portal system and reach their site of action, the pars distalis of the adenohypophysis, via the bloodstream (so-called neurovascular chain). The presence of LRH in hypophyseal portal blood has been demonstrated in rats (FINK, 1967). There is no direct nervous connection between hypothalamus and adenohypophysis, so that active substances from the hypothalamus can only reach the adenohypophysis via the neurovascular chain. The integrity of the hypophyseal portal system is therefore essential for optimal function of the adenohypophysis (POPA, 1930). It is still not known whether RH merely cause the liberation of gonadotropins, or whether they are also necessary for their formation. It is generally assumed that the latter is true.

The content of FRH in the hypothalamus is increased in the castrated animal. It decreases after administration of estrogens. It has also been possible to show that sexual steroids and ovulostatics influence the content of FRH and LRH in the hypothalamus (MARTINI, 1968). This shows that the feedback mechanism regulating the release of FSH and LH passes through the hypothalamus.

Not only releasing elements, but also inhibitory factors are produced in the hypothalamus. Thus, transplantation of the hypophysis into the region of the temporal lobe, for example, leads to continuous secretion of LTH in female rats, and thus to persistent corpora lutea (EVERETT, 1956). It is presumed because of this that there is a hypothalamic LTH- or prolactin inhibitor factor (PRIH) which inhibits prolactin release (MEITES, 1963). In 1965, PRIH was shown to be present in hypothalamic extracts of sheep, cow and pig (SCHALLY, 1965). In addition, radioimmunological studies have shown that porcine hypothalamic extracts reduce the prolactin level in sheep (ARIMURA, 1969). PRIH withstands boiling, diffuses through semipermeable membranes and has a molecular weight between 1000 and 2000. In contrast to the anterior pituitary cells producing FSH and LH, in which secretion mainly occurs only after hypothalamic stimulation, formation and liberation of prolactin appear to be inherent abilities of these cells. In the presence of intact hypothalamo-hypophyseal connections, prolactin secretion is inhibited by the hypothalamic PRIH.

The amenorrhea with galactorrhea sometimes associated with low FSH values and observed in women with Chiari-Frommels' disease and Forbes-Albright syndrome, in pseudocyesis and during medication with chlorpromazine and reserpine etc. (p. 592) imply the loss of hypothalamic influence on the adenohypophysis. Hypothalamic stimulation of the pituitary cells producing FSH and LH and the central inhibitory action on prolactin production are absent at the same time.

Section of the pituitary stalk results in loss of ovarian function. The recovery of ovarian function is parallel to regeneration of the hypophyseal portal system. As soon as this has regenerated, ovarian cycles return and the animal becomes fertile again. If, however, regeneration of the hypophyseal portal system is definitely prevented, for example mechanically, ovarian atrophy occurs. Destruction of the eminantia mediana produces the same effect.

A small degree of FSH and LH secretion appears, however, to occur independently of the hypothalamus. This was deduced from animal experiments. If a large number of pituitaries are transplanted into a hypophysectomized animal, for example into the eye, the ovaries do not atrophy and ovarian function is restored (GITTES, 1965).

Pituitary transplants into hypophysectomized animals (HARRIS, 1957) have shown, further, that the function of the anterior pituitary is dependent on sexual differentiation and the state of maturation of the hypothalamus. If the hypophysis of a male is transplanted into a hypophysectomized female rat, it functions as a female hypophysis, meaning that it retains its pluripotential ability and functions according to female hypothalamic stimulus. If the hypophysis of a prepuberal animal is transplanted into a hypophysectomized, sexually mature animal, it functions like the hypophysis of a sexually mature animal.

To summarize, central ovarian regulation has the following anatomical-functional structure:

the adenohypophysis lacks the ability to form and secrete significant amounts of FSH and LH independently of the hypothalamus. On the other hand, it possesses an inherent capacity to produce and secrete prolactin.

Two regulatory systems are localized in the hypothalamus. They control the formation and release of pituitary gonadotropins:

a) A primary system which exerts a continuous stimulatory effect on formation and release of FSH and LH, and which has a continuous inhibitory action on formation and secretion of prolactin (tonic center). It is localized in the region of the periventricular area and eminentia mediana. Stimuli are transmitted via neurosecretions which are described as releasing hormones (RH). The route of transmission is neurovascular, and the "trade-center" is the eminentia mediana.

b) A secondary system which inhibits the primary center and induces cycles in the woman (cyclic center). Its functional state is influenced by proprioceptive and exteroceptive stimuli. It lies in the rostral hypothalamus just below the paraventricular nucleus.

3. Regulation Mechanism

Hypothalamus, hypophysis and ovaries form a neurohormonal *functional circle* which is self-regulating, adjusting hormone release to momentary needs. Regulation functions according to the feedback, push-and-pull principle also termed the so-called cybernetic principle (Fig. 40). The feedback mechanism is based on the reciprocal relation between sexual steroids and gonadotropins.

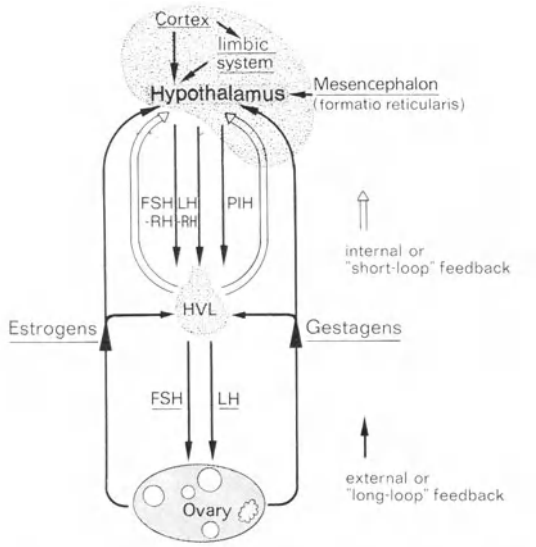


Fig. 40. Diagram of the neurohumeral function circuit: cortex-hypothalamus-hypophysis-ovary

The following main stages occur (ROTHCHILD, 1967): FSH leads to maturation of the follicle. Together with LH, it results in the formation of estrogens, particularly in the thecal cells of the developing follicle. The increasing estrogen secretion during the proliferative phase stimulates the secondary system situated in the anterior hypothalamus, so that the release of FRH is inhibited while LRH release is stimulated at the same time. This results in a reduction of FSH secretion and a simultaneous rapid rise in LH secretion. This then leads to terminal growth of the graafian follicle and to ovulation. The fall in the levels of estrogens after ovulation causes a new rise in FSH release due to the decreased stimulatory effect of estrogens on the hypothalamus. The ruptured follicle is luteinized by LH, whereby progesterone, acting as an antiestrogen, inhibits LH stimulation caused by the rising estrogen secretion. It has not been proved in the human whether the action of progesterone is analogous to that of estrogens, influencing LRH secretion centrally via the rostral hypothalamic inhibitory center. The same can be said of the direct influence of sexual steroids on the adenohypophysis. Regression of the corpus luteum menstruationis and the diminished estrogen production resulting from this together lead again to increased FSH secretion over both hypothalamic centers, and thus to a new ovarian cycle.

From the facts stated so far, it can be deduced that the influence exerted by sexual steroids on the hypothalamus is dependent on the chemical nature of these steroids, their dosage, and the duration of action, and also on the functional state of the hypothalamus ifself. Among the naturally occurring sexual steroids, estrogens exert the strongest central inhibitory effect, which surpasses the action of progesterone by far. In contrast, synthetic progestagens (19-nortestoid and 17α-acetoxy-progesterone) have a pronounced central inhibitory effect. This is exploited therapeutically to inhibit ovulation (p. 568). Estrogens in physiological doses can release ovulation, probably by causing short-term increases in LH secretion. In the same way, pre-ovulatory formation of progesterone is thought to favor ovulation in the human. These pre-ovulatory influences are of short duration. The long-lasting effect of estrogens (and androgens) is analogous to the action of higher doses causing the release of gonadotropins to be inhibited. The long-lasting action of progesterone prevents the liberation of LH in the pre-ovulatory phase. In rats treated with progesterone, ovulation only occurs after stimulation of the eminentia mediana. It is probable that treatment with progesterone results in an elevated LH concentration in the adenohypophysis due to inhibition of the release of LH.

High doses of estrogens and progesterone almost completely suppress secretion of pitui-

tary gonadotropins. A physiological suppression of this type is observed during pregnancy.

The reaction capacity of the hypothalamus is influenced by the functional state of the limbic system (p. 555). The inhibiting action of certain drugs on hypothalamic nuclei must also be mentioned. These drugs are morphine, reserpine, atropine, pentobarbital and chlorpromazine. Their site of action has not been confirmed. Interruption of endogenous stimuli from the reticular formation is under discussion. According to recent investigations (KELLER, 1968), clomide also exerts its anti-estrogenic action centrally in man. If estrogens are given to women with hypergonadotropic amenorrhea, there is a reduction of gonadotropin excretion (FSH and LH). On the other hand, if clomide is given together with estrogens, there is a significant rise in LH release.

In addition to the indirect, external feedback mechanism already described, there may be a direct internal feedback (the so-called short feedback mechanism) (DAVID, 1966), between pituitary and hypothalamus (SZONTAGH, 1964).

The peripheral regulatory system, the autonomic nervous system and the peripheral endocrine organs interfere with the centrally controlled cybernetic system. Loss of this intervention leads to equilibrium disorders. The peripheral regulatory system is composed of various parts – the ovaries as chief site of formation of estrogens, the liver as site of breakdown and conversion, kidneys and intestine as site of excretion, tissue as depot, and blood as transport agent. This peripheral system influences the blood level of sexual steroids considerably. Finally the autonomic nervous system determines circulation and course of

Table 22

International name	Synonyms	Chemistry and physical data (ODELL, 1964)	Origin (HERLANT, 1966)	Effect
Hypophyseal gonadotropins				
FSH	Follicle-ripening hormone, follicle-stimulating hormone, prolan A, gonadotropin I, thylacentrin, gametokinetic hormone, epithelial factor	Glycoproteid: protein + mannose. Mol. wt: 41000. Soluble in water, soluble in trichloroacetic acid	γ cells (amphophile = chromophobe cells)	Growth of secondary and tertiary follicles
LH = ICSH = luteinizing = interstitial cell stimulating hormone	Lutinizing hormone, corpus luteum-ripening hormone, metakentrin, ovulation hormone, prolan B, gonadotropin II	Glycoproteid: protein + mannose. Mol. wt: 30000. water-soluble, insoluble in trichloroacetic acid	δ cells (hypocyanophile cells)	With FSH: estrogen synthesis and release of ovum; formation of corpus luteum
LTH = LMTH = luteotropic hormone, luteomammatropic hormone	Luteotropic hormone, prolactin luteotropin, lactation hormone, lactogenic hormone, galactin, mammotropin, gonadotropin III	Simple protein. Mol. wt: 32000. hardly soluble in water, soluble in slightly acidic methyl and ethyl alcohol	α cells (eosinophilic cells)	In rodents: stimulates endocrine function of corpus luteum, preserves corpus luteum
HMG = human menopausal gonadotropin	Menopausal gonadotropin, castrate's gonadotropin	Glycoproteid: protein + hexose. Mol. wt: 36000. water-soluble	γ + δ cells	
Placental gonadotropins				
HCG = human chorionic gonadotropin	Chorionic gonadotropin, placental gonadotropin, pregnancy urine hormone (PU), anterior pituitary-like hormone (APL)	Glycoproteid: Mol. wt: 29000–30000. water-soluble	Trophoblast	Similar to LH
PMS = pregnant mare's serum gonadotropin	Serum gonadotropin, equine gonadotropin	Glycoproteid: protein + galactose. Mol. wt: 30000 water-soluble	Trophoblast or decidua	Similar to FSH

reaction attitude of the peripheral endocrine glands and their reacting organs by direction of the effect and tone.

4. The Pituitary Gonadotropins (Table 22)

The ovaries of a non-pregnant, sexually mature woman are controlled by pituitary gonadotropins, follicular stimulating hormone (FSH) and luteinizing hormone (LH), also known as the interstitial cell-stimulating hormone (ICSH). It has only recently been successfully proved, after numerous objections, that FSH and LH are two different pituitary gonadotropins. The activities of the two hormones can be more or less completely separated by chromatography (REICHERT, 1964; ALBERT, 1965), and they also show differences in metabolism (p. 560) (KELLER, 1966). Trypsin destroys 99% of LH, whereas FSH is not affected. There are still no FSH and LH preparations which are absolutely chemically and immunologically pure. Only highly purified pituitary or urinary FSH and LH are available (ROOS, 1964). Thus, NIH-FSH-S_1 contains only 0.6% LH (PARLOW, 1961). In contrast to the situation in the rodent, the luteotropic hormone (LTH) seems to have no effect on ovarian regulation in the human. FSH and LH are sufficient to produce a biphasic cycle with normally functioning corpus luteum in hypophysectomized women (GEMZELL, 1964). Nor is LTH identical to prolactin in the woman.

Structure. FSH and LH are glucoproteins (GÖSCHEL, 1960) containing the carbohydrate mannose. Apart from containing glucosamine, sialinic acid is bound terminally to the protein (Table 23). This forms an integral component of the biological potency of these two hormones. Enzymatic splitting of sialinic acid by neuramidase leads to a loss of at least 97% of the activity. Splitting off of mannose also results in a definite loss of activity. Although pituitary FSH and urinary FSH have the same qualitative composition and clinical action, they appear to differ in molecular weight (ROOS, 1967) (Table 23).

Table 23. Chemical and biological properties of human FSH in hypophysis and urine. (After P. ROOS, 1967)

	Hypophyseal FSH	Urinary FSH
Molecular weight	41000	28000
No. of amino acids	254	208
Residues per mol		
Hexose	39	14
Glucosamine	30	8
Sialinic acid	8	2
Biological activity IU/mg	14000	780
Yield	2 µg/hypophysis	1 µg/liter urine

a) Production and Plasma Content

Hypophyseal gonadotropins are formed in the anterior pituitary. It is still not clear what type of cells synthesize them (HERLANT, 1966). Whereas the fetal hypophysis appears to contain very small amounts of gonadotropins, there is none present in children before the 6th year (Table 24). On the other hand corticotropin and somatotropin are present in the infantile hypophysis in similar concentrations to those in the adult.

The lack of pituitary gonadotropins during infancy is substantiated by the observation that gonadotropins cannot be demonstrated in the urine until 3–4 years before menarche. The appearance of pituitary gonadotropins in the urine before the 9th year of life is described by JOHNSON (1959) as pathologic. They should, however, be present in the urine not later than the 13th year.

FSH and LH content in the hypophysis rises during the individual phases of life of the woman right into senility, except during pregnancy.

During pregnancy, the gonadotropin content in the pituitary is low, due to the high level of sexual steroids. Conversely, the pituitary contains the largest amounts of gonadotropins after the menopause (Tables 24, 25). Release and probably also formation of gonadotropins results from the influence of releasing hormones of the hypothalamus (p. 555).

Investigations on the *transport* of gonadotropins *in the blood* have yielded very variable results. It is possible that they are transported in Cohn fractions II and III, which include β-lipo-proteins and γ-globulins. In sexually mature women, plasma values of total gonado-

Table 24. Total gonadotropin content (TGA) of human hypophyses. (After BETTENDORF, 1962)

	No. of hypophyses processed	TGA[a] per mg dry weight	TGA[a] per hypophysis
Fetuses	6	0.5	1.6 (0–4.0)
Children up to 5 years	6	neg.	neg.
Women >60	6	11 (5–17)	1360 (465–2320)
Pregnant women	4	0.2 (0.1–0.3)	36 (17–68)

[a] HMG units (HMG-20A). Extremes of range given in brackets.

tropic activity vary between 0.6–3 mg of the 2nd international reference preparation (2nd IRP-HMG) per liter plasma. In the postmenopause these values are 20 times higher, lying between 10–50 mg of the 2nd IRP-HMG per liter of plasma (KELLER, 1965; MIDGLEY, 1968) (Table 26).

Table 25. LH content of human hypophyses (After RYAN, 1962)

	No. of hypophyses processed	LH per g wet weight	LH per[a] hypophysis
Children up to 5	4	0.37 (0.05–0.55)	0.034 (0.009–0.052)
Women (20–40 years)	5	2.48 (0.24–5.14)	1.25 (0.16–2.21)
Women in premenopause	2	3.70 (3.16–4.23)	1.92 (1.31–2.53)
Women in postmenopause	4	7.64 (2.04–15.13)	3.45 (1.19–5.46)

[a] In mg NIH-LS.

Table 26. Normal gonadotropin values in plasma in woman

Stage of life	TGA mg-eq. 2 IRP per liter	FSH IU per liter	LH IU per liter
Sexual maturity	0.5–2.4[a]	6.2–10.8[b] (Ovulations-max. 21.5)	7.4–13.2[b] (Ovulations-max. 47.8)
After menopause	10–46[a]	80–200[a]	50–200[a]

[a] P. J. KELLER (1966).
[b] A. R. MIDGLEY (1968).

b) Metabolism and Excretion

Very little is known about the metabolism. The functional state of the liver does not seem to influence gonadotropin levels in the blood and urine. Gonadotropins are excreted through the kidneys, although these hormones are highly molecular glycoproteins. Comparative studies in plasma and urine have shown that the average renal clearance for total gonadotropic activity is 0.17 ml/min (APOSTOLAKIS, 1960), 0.58 ml/min for FSH, and 0.14 ml/min for LH (KELLER, 1966). It is 0.95 ml/min for HCG (LORAINE, 1950). Only a small proportion of plasma gonadotropins are excreted through the kidneys. Most of the secreted gonadotropins are probably inactivated in some still unknown manner. The obviously different excretion ratios for FSH and LH also add to the evidence that they are two different substances. These ratios also explain why urinary and plasma gonadotropins are qualitatively different in composition (Table 23). Gonadotropins are not demonstrable in the urine until 3–4 years before menarche. In adolescence there is an irregular excretion pattern with isolated FSH and LH peaks until regular biphasic cycles are established. Excretion values of gonadotropins exhibit a characteristic course in the sexually mature woman (ROSEMBERG, 1965; MCARTHUR, 1958; FUKUSHIMA, 1964). Both gonadotropins are demonstrable throughout the cycle, LH showing a higher peak than FSH at the time of ovulation (1st to 6th day and 1st to 2nd day respectively after fall in temperature). At mid-cycle, LH levels in the urine reach 40 IH/24 h (Fig. 41). The total gonadotropic activity (TGA) in the 24-hour urine specimen varies between 0.4–2 mg of the 2nd IRP-HMG (Table 27).

A considerable change in the FSH/LH quotient can arise in pathologic endocrine states (Table 28). These are only temporary values and other investigators have obtained different results. The normal LH titer and the almost absent FSH titer are striking findings in precocious puberty, and it can be assumed from this that premature stimulation of the

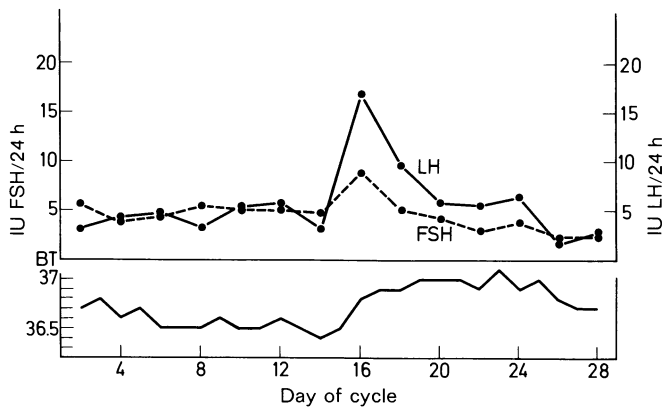


Fig. 41. Excretion curves for FSH and LH during the normal biphasic cycle (after KELLER, 1970). (Plotted from pooled values in 7 cycles)

Table 27. Normal gonadotropin excretion values in woman. (After KELLER, 1969)

Stage of life	TGA mg-eq./24 h	FSH IU/24 h	LH IU/24 h	Characteristics
Childhood	0.2	2	3	No gonadotropins demonstrable until 3–4 years before menarche
Adolescence	0.2–1.0	2–8	3–10	Irregular pattern of excretion with separate peaks for FSH and LH
Cycle	0.4–2.0	4–15	2–8	Mid-cycle rise in FSH and LH, LH up to 40 IU
Pregnancy	–	–	–	Not determinable due to presence of HCG, probably secretion of FSH and LH is copious
Lactation	0.4–2.0	4–10	2–8	Situation similar to that during cycle, isolated LH peaks possible
Premenopause	3.0–40	40–400	15–90	Rapid rise of FSH values, less pronounced rise in LH values
Postmenopause	3.0–15	40–200	10–60	High gonadotropin values persisting into senium in absence of cycles

Table 28. FSH and LH excretion in endocrine diseases (KELLER, 1968)

Clinical diagnosis	Cases	FSH excreted IU/24 h	LH excreted IU/24 h	FSH/LH
Pubertas praecox	3	1.3–2.0	1.8–3.7	0.5–0.8
Fertile eunuchs	3	2.4–8.3	5.9–9.5	0.4–0.9
Hypophyseal adenoma	1	2.5–3.5	4.3 4.5	0.6–0.8
Stein-Leventhal syndrome	3	6.0–9.9	5.5–16	0.6–1.5
Klinefelter's syndrome	2	81–101	44–69	1.5–2.3
Ovarian dysgenesis	2	38–44	21–22	1.8–2.1
♂ Castrates	1	28–31	5.1–9.2	3.4–5.5
Turner's syndrome	3	73–110	20–22	3.6–5.0
♀ Castrates	3	25–45	3.7–5.1	5.1–8.8

48-h to 144-h aliquots urine. Extraction after ALBERT. Determination of FSH after STEELMAN and POHLEY. Determination of LH by ascorbic acid depletion test after PARLOW. Standard: 2 IRP HMG.

sexual steroids is due only to LH. The relatively high production of LH in fertile eunuchs indicates peripheral failure and not primary pituitary insufficiency. Whereas the activity of both FSH and LH is elevated in ovarian dysgenesia, it is predominantly FSH which is elevated in cases of male gonadal dysgenesia and in castrated women.

During the climacteric, FSH values rise rapidly and LH to a lesser extent. These values remain high right into senility when cycles disappear (Tables 26, 27). Both values fall again slightly after the 70th year.

c) Biological Action

One action of FSH is to stimulate follicular maturation. Estrogens are produced under the combined influence of FSH and LH, predominantly in the thecal cells of the growing follicle. LH also stimulates the 20- and 22-hydroxylation of cholesterol, producing pregnenolone in the process. (ISCHII, 1963; MASON, 1961). Increasing amounts of estrogen act on the hypothalamus and probably cause a decline in FSH secretion by means of the feedback mechanism. The increasing estrogen titer may stimulate the release of LH. The terminal spurt of growth of the Graafian follicle and ovulation are due to the rapid increase in LH liberation. The number of follicles which reach maturity and the rate of maturation seem to be constant for each species (GEMZELL, 1969). Administration of additional FSH increases the number of mature follicles, but the speed of maturation is unchanged. In order to produce the number of fertile ova specific to any one species, the amount of FSH secreted by the anterior pituitary must be constant. In the human, only one follicle of the original group of maturing follicles reaches full maturity and ovulation; the others become atretic. The mechanism of this selection is not known.

The ruptured follicle becomes luteinized under the increasing influence of LH. Synthesis of progesterone and estrogens is stimulated in the corpus luteum. Progesterone inhibits LH secretion, either by a direct central action or by its antiestrogenic effect. This leads to regression of the corpus luteum about 10 days after ovulation in the absence of a pregnancy. The fall in the estrogen levels causes the feedback mechanism to fail and there is a new

increase in FSH during menstruation. LH secretion, however, remains low, since the low estrogen production is insufficient for stimulation of LH secretion. Thus, a new cycle is started.

In recovery tests in young women with primary amenorrhea and low urinary excretion of FSH it was possible to recover 12.8–20.8% (average 17%) of the FSH administered from the urine (GEMZELL, 1969). During a normal 28-day cycle, about 360 IU FSH (2nd IRP-HMG) can be found in the urine. About 170 IU FSH are found during the first days of the cycle. The hypophysis of a normally ovulating woman secretes about 2000 IU FSH during a four-week cycle, about half of it before ovulation (GEMZELL, 1969).

d) Estimation

In contrast to the estimation of HCG, that of pituitary gonadotropins in the urine and plasma is still associated with considerable difficulty. Pituitary gonadotropins can be detected by

Table 29. Most usual biological methods for the estimation of gonadotropins. (After KELLER, 1971)

Test	Specificity	End point	Duration of test (days)	Sensitivity	Precision
Mouse uterus test (KLINEFELTER, 1943) (MU test)	TGA	Weight of uterus in infantile mice	3	0.03 mg-eq. 2nd IRP	0.06–0.15
Rat ovary test (RO test)	TGA	Weight of ovaries in infantile rats	3.5	0.2–0.4 mg-eq. 2nd IRP	0.10–0.24
Augmentation test after STEELMAN and POHLEY (1953)	FSH	Weight of ovaries in HCG-stimulated infantile rats	3.5	1–2 IU FSH	0.10–0.24
Augmentation test after BROWN (1955)	FSH	Weight of ovaries of HCG-stimulated infantile mice	3.5	0.5–1 IU FSH	0.20–0.50
Ventral prostata test (VRP test) (GREEP 1941)	LH (ICSH)	Weight of ventral flap of prostata of infantile, hypophysectomized rats	4.5	0.4–0.8 IU LH	0.15–0.40
Ovarian ascorbic acid depletion test after PARLOW (1958, 1960) (OAAD test)	LH (ICSH)	Fall in ascorbic acid in ovary of infantile pseudopregnant rats	1	0.3–0.6 IU LH[a]	0.10–0.28

[a] When NIH LH is used as the standard preparation the sensitivity is approximately 10–12 times higher.

Table 30. Most usual methods for extraction of gonadotropins from urine and plasma. (After KELLER, 1971)

Method	Principle	Processing time (hours)	Yield
1. Urine			
Kaolin and acetone method after ALBERT (1955)	Adsorption of gonadotropins onto kaolin at acid pH, elution with ammoniac and precipitation with acetone	1–1.5	50–200 mg/24 h
Kaolin and acetone method after LORAINE and BROWN (1954)	As above	2–3	150–500 mg/24 h
Tannic acid method after JOHNSON (1959)	Precipitation of gonadotropins in unacidulated urine with gallic acid with addition of Hyflo-Supercel, elution with ammoniacal alcohol, precipitation with alcohol	1–1.5	20–100 mg/24 h
2. Plasma			
Acetone precipitation after APOSTOLAKIS (1960)	Precipitation of total proteins with acetone, dialysis against physiological saline	1	60–80 mg/ml
Alcohol and ammonium acetate method after KELLER and ROSEMBERG (1965)	Precipitation of total proteins with alcohol, extraction with ammonium actetate and alcohol, reprecipitation with alcohol	1–1.5	5–12 mg/ml

biological, immunochemical or radioimmunological means.

Biological methods are now principally used for routine investigations (KELLER, 1963). They are based mainly on stimulation of the genital organs of rats and mice (Table 29) and involve two steps: concentration of gonadotropic substances from urine or plasma (Table 30), and biological testing of the extracts (Table 29). Since the concentration of pituitary gonadotropins is low in urine and even lower in plasma, accumulation is necessary for the biological estimation. We have found that among the numerous extraction methods (Table 30) from urine, the *kaolin-acetone method* after ALBERT (1955), modified after HEINRICHS and EULEFELD (1960), has proved most suitable for routine laboratory work (Table 31). The average recovery is 93.7%, and the method is quick, cheap and simple to carry out.

Immunochemical methods, in which gonadotropins are estimated with the aid of specific immune antibodies, are increasing in importance. This is especially applicable to estimations of HCG and LH, since there is a cross-reaction between HCG and LH. Indeed, the estimation of LH activity by means of an antiserum to highly purified pituitary LH gives practically identical values to those obtained when antiserum to HCG is used instead. Immunological measurement of LH in the urine is very sensitive, simple, and quick. It gives a reliable picture of the excretion pattern for LH (Fig. 42).

Table 31. Extraction of hypophyseal gonadotropins from urine after ALBERT. (After KELLER, 1971)

Acidulation of the 24-h urine to pH 4.5 with glacial acetic acid and addition of 20 g kaolin. Mix with magnetic stirrer for 3 min.
↓
Filter of the urine in automatic rapid filter (Spincraft) and was the residue with 2 liter water at pH 4.5.
↓
Elution of the gonadotropins with 100 ml 2 mol NH_4OH pressed through twice. Final wash with 30 ml distilled water.
↓
Adjust to pH 5.5 with glacial acetic acid and extract with 2 volumes acetone.
↓
Leave to standard at 4°C for 30 min, centrifuge, dry the precipitate.

However, there is still no proof that the reaction for LH is specific, and that augmentation factors are not determined at the same time. LH measured biologically is not identical to LH estimated immunologically. It is thus possible that the biological extraction necessary alters the biological action of the gonadotropins. Obtention of a genuinely pure preparation for the production of specific antibodies is the greatest problem in immunological methods.

Immunochemical assay of FSH in the urine has recently also become possible. In the serum,

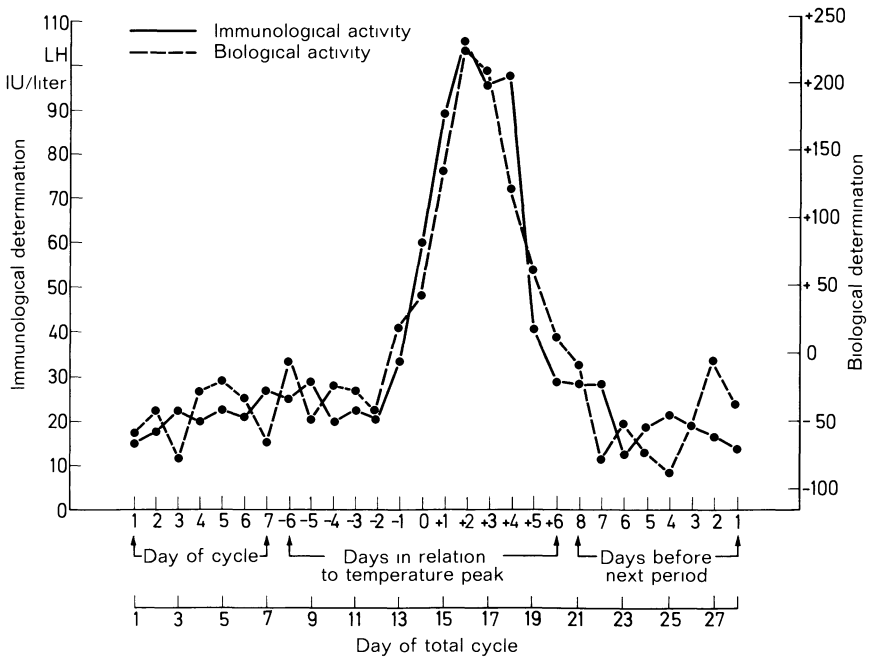


Fig. 42. Excretion curve for LH during a normal cycle, plotted from values determined by a biological (ventral prostate test) and an immunological method. (After WIDE, 1966; from MCARTHUR and INGERSOLL, 1958)

plasma, and in certain conditions in the urine, FSH and LH can be estimated by *radioimmunoassay*. As this is a rather sophisticated method it is still performed only in a few specialized laboratories.

Chemical methods, which are still based on the demonstration of glucoproteins, are not specific enough. Further progress is necessary before anything more can be learnt about the chemical structure of gonadotropins. Lack of a chemical method for the quantitative estimation of gonadotropins and of chemically pure gonadotropin are the main reasons why there is still no reliable, generally recognized weight unit for gonadotropins.

The standards in use for pituitary gonadotropins and the conversion factors are presented in Table 32. The 2nd international reference preparation (2nd IRP) is the standard preparation used at present. It is obtained from postmenopausal urine, and one ampoule contains 5 mg active material or 40 IU FSH or LH.

Table 32. Usual standards for hypophyseal gonadotropins. (After KELLER, 1971)

Preparation	Application	Unit	Conversion
HMG 24 (1st IRP)	TGA	mg-eq.	1 mg ~ 0.06 mg 2nd IRP
	FSH	mg-eq.	1 mg ~ 0.15 IU FSH
	LH	mg-eq.	1 mg ~ 0.50 IU LH
2nd IRP	TGA	mg-eq.	1 mg ~ 8 U ~19 mg HMG 24
	FSH	IU	1 IU FSH: ~0.045 mg NIH FSH S_1 ~7 mg HMG 24
	LH	IU	1 IU LH: ~0.015 mg NIH LH S_1 ~2 mg HMG 24
NIH FSH S_1	FSH	mg-eq.	1 mg ~ 22 IU ~150 mg HMG 24
NIH LH S_1	LH	mg-eq.	1 mg ~ 67 IU ~135 mg HMG 24

Conversions for LH are based on the ventral prostata test. Other factors must be considered if the OAAD is used.

e) Therapeutic Use

Due to their ability to stimulate follicular growth and to induce ovulation, pituitary gonadotropins are used in the treatment of anovulatory sterility. Originally, extracts from animal pituitaries and from the serum of pregnant mares (PMS = pregnant mare's serum) were used as FSH preparations. The results obtained with these extracts were, however, disappointing, since these heterologous gonadotropins showed an antigenic effect in man. So-called antihormones are formed, which weaken or inhibit gonadotropic activity. In addition, the action of these heterologous proteohormones is probably inconstant in man due to species-specificity. Quite often allergic reactions were produced. Ovulation was successfully induced in women with amenorrhea for the first time in 1958, by administration of FSH from human pituitaries with human chorionic gonadotropin (HCG) (GEMZELL, 1958).

It was shown subsequently, in 1960, that FSH obtained from the urine of postmenopausal women, was clinically active (LUNENFELD, 1960). This substance was given the name human menopausal gonadotropins (HMG) and came onto the market in the purified form in 1964, under the name Pergonal (75 IU FSH + 75 IU LH) (DONINI, 1964). Humegon (75 IU FSH) is also obtained from postmenopausal urine and is used for the same purpose.

Ovulation release due to human FSH and HCG is dependent on various factors (GEMZELL, 1969):

1. the ratio of FSH to LH in the preparation used;
2. the total dose of FSH/LH and HCG;
3. the time at which FSH/LH and HCG are given.

The ratio of FSH to LH can, seemingly, vary between 1 : 2 and 2 : 1 with no significant loss of activity. From recovery experiments with physiological doses it has been calculated that 75–100 IU of FSH must be administered daily for 8–10 days to produce ovulation (p. 562). If no reaction occurs, the daily dose can be increased to 250 IU, although the risk of overstimulation increases rapidly (p. 600, 630). There is no necessity to separate human LH for therapeutic purposes. Large amounts of chorionic gonadotropin (HCG) occur in pregnant urine, and the luteinizing effect of this HCG is adequate. HCG is usually given in single doses; as a rule, 5000 IU are given daily for 3 days, the first dose of HCG usually coinciding with the last of FSH. The results obtained with this method are very good with the right choice of patients (GEMZELL, 1958, 1963, 1966, 1967, 1969; TAYMOR, 1963; ROSEMBERG, 1964; BETTENDORF, 1964; VAN DE WIELE, 1965; KISTNER, 1966). (Refer to p. 600, 630 for more about treatment.)

E. Ovulation

Ovulation is the central event in a normal biphasic ovarian cycle. During this process, the fertile ovum leaves the ovary and reaches the tubal ampulla by way of the collecting mechanism.

The processes responsible for ovulation are the same in all spontaneously ovulating animals (e.g. mice, rats) and in women. The preovulatory increase in the ovarian secretion of progestin (EVERETT, 1944) causes the peak of preovulatory LH secretion due to increased LRH secretion (MCARTHUR, 1959). This peak in turn leads to the terminal spurt of growth of the Graafian follicle and to ovulation.

Anatomy, endocrinology, and central regulation of ovulation are discussed on p. 512ff., 544ff.

1. Influence of Thyroid Gland and Adrenal Cortex on Ovulation

A high percentage of female patients with thyroid dysfunction, whether hypothyroidism or hyperthyroidism, suffer from anovulatory cycles with oligomenorrhea or amenorrhea and from dysfunctional hemorrhage depending on the severity of the thyroid disorder (SCHNEEBERG, 1967). The pathophysiology underlying ovarian dysfunction in these conditions is, however, not exactly known at present. Experiments in rabbits, mice, rats and baboons show that thyroid hormones can intervene at various stages in the complex mechanism of ovulation. Thus the injection of thyroxine leads to an elevated concentration of thyroxine in the paraventricular nucleus and eminentia mediana (FORD, 1958). Surgical removal or medical inactivation of the thyroid gland causes FSH secretion to be increased and LH secretion to be reduced, resulting in follicular persistence and endometrial hyperplasia (CHU, 1945), and also in increased sensitivity of the ovaries to exogenous gonadotropins (FLUHMANN, 1934; MANDL, 1957). It is assumed that thyroxine stimulates LH secretion and inhibits formation of LH (CHU, 1945).

Whereas there is no doubt that there is an etiological connection between thyroid dysfunction and anovulation, it is doubtful whether there is any such connection in sterile women who are euthyroid. Much has been written about the value or otherwise of treating female infertility with thyroid hormones (ENGLE, 1959; KUPPERMANN, 1959; KOTZ, 1961; STARR, 1962, 1966; ROGERS, 1963; LLOYD, 1964; SCHNEEBERG, 1967). Although ovulation induction by thyroid hormones has not so far been statistically proved in sterile euthyroid patients, there are still people who support this form of treatment of female sterility (ENGLE, 1959; KUPPERMANN, 1959; STARR, 1962). However, since the introduction of human gonadotropins and antiestrogens into the treatment of anovulation, the empiric treatment with thyroid preparations for female sterility has not been considered so important. In our opinion this form of treatment is not justified in clinically euthyroid women in whom laboratory findings are normal. The objection that subclinical states of hypothyroidism may be overlooked is in our opinion not valid.

It has long been known that there is a functional relationship between *adrenal cortex and ovary*. Disturbances of adrenocortical function associated with increased production of sexual steroids, such as Cushing's syndrome and the adrenogenital syndrome, are connected with ovarian dysfunction particularly often. It can be shown experimentally that adrenalectomized rats have no ovarian reaction to injected gonadotropins. This observation is equivalent to the clinical finding of amenorrhea in female Addisonian patients.

Despite numerous studies (JEFFERIES, 1966) the exact nature of the mutual influence between adrenal cortex and ovary are still not clear. The increased androgen production in Cushing's syndrome and the adrenogenital syndrome suggests that this may interfere with ovarian function via the hypothalamus and pituitary. This conjecture is supported by the observation that numerous women with ovarian dysfunction show signs of acne, and hirsutism and have a slightly higher 17-ketosteroid excretion than normal. It is, however, true that women with abnormally high androgen production can be fertile, and also that even slightly higher androgen production than normal can lead to considerable ovarian dysfunction. It is possible that the increase in adrenal estrogen production which is often found at the same time is etiologically involved in the ovarian dysfunction.

Prolonged cortisone treatment can lead to normalization of ovarian function in some cases. JEFFERIES (1963) reported on 104 women with ovarian dysfunction, in whom gonadotropin excretion was normal or low and in whom there was no tumor or primary pituitary disturbance. These women received 10 to 20 mg cortisone daily for 6 months. Ovarian function improved in 4 out of 5 patients, and 2 of every 3 sterile women conceived when the husband's semen was normal.

2. Proof of Ovulation

Despite all efforts, there is still no reliable and simple method of estimating the exact time of ovulation. Even success of artificial insemination at the first attempt and pregnancy arising after a single act of intercourse permit only approximate dating of ovulation, since the lives of the ovum and sperms are of un-

certain duration. Exceptionally, ovulation can be visually observed during culdoscopy, culdotomy or laparotomy, but these methods are not suitable for practical purposes. Biopsy of the corpus luteum for the histological assessment of its age (HARTMANN, 1955) is only possible in special cases and hardly offers any more information than is obtained from the endometrial biopsy (p. 566).

The occurrence of ovulation can be shown by a number of other methods, some of which are indirect and not entirely reliable. Pregnancy is the only certain proof of ovulation. In all other cases, several indirect methods must be employed together to show that ovulation has occurred. Since none of them is fully reliable, the result of any single test is a mere indication. All the tests are based on the formation of progesterone in the corpus luteum.

a) Pregnanediol Excretion in the Urine (Fig. 43)

Less than 1.5 mg pregnanediol is excreted in 24 hours until shortly before the fall in urinary estrogens and shortly after the basal temperature rises (KLOPPER, 1957). This pregnanediol derives from adrenal progesterone (residual progesterone). On day 2 or 3 after the temperature has increased, progesterone excretion rises rapidly and reaches its peak 5–7 days before the onset of menstruation. A maximum of about 5 mg pregnanediol is excreted in 24 hours in the presence of one corpus luteum (KLOPPER, 1957), but GEMZELL and ROOS (1966) calculated significantly higher values in the cases of multiple pregnancies, indicating that several corpora lutea had developed at the same time. However, greatly increased pregnanediol values in the urine are no definite evidence that ovulation has occurred or that a corpus luteum has been formed. This fact has been confirmed by observation of cases in which a greatly elevated pregnanediol excretion was found to be due entirely to luteinization of the thecal cells of several follicular cysts (GARCIA, 1967).

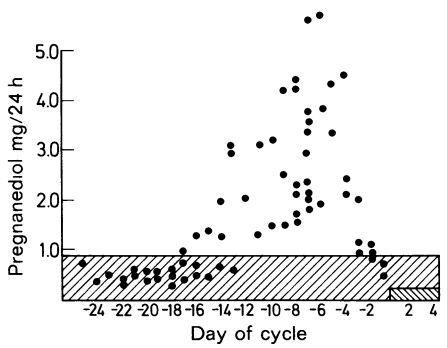


Fig. 43. Excretion of pregnanediol in 18 women during a biphasic cycle. The numeric values refer to the number of days before the beginning of the next period. (After GUAL, 1966)

b) Endometrial Biopsy (p. 625)

The study of so-called diagnostic smears from endometrial strips obtained from outpatients is a relatively reliable indirect method of determining histologically whether ovulation has occurred or not. This method is well suited for fixing the time of ovulation. It is best to take the biopsy from the fundus of the uterus on the 9th day of the hyperthermic phase. Errors of interpretation are reduced by these measures. In contrast to pregnanediol excretion, which can vary greatly from one person to another (Fig. 43), the histological picture of the endometrium changes uniformly and quite regularly under the influence of progesterone (ROCK, 1937; HERTIG, 1946; NOYES, 1950) (Fig. 29). An experienced investigator can determine the cyclic phase to within 48 hours from the degree of secretory transformation (p. 540), which allows the time of ovulation to be assessed quite accurately (NOYES, 1950). Reproducibility and accuracy are good with this method (NOYES, 1953), and endometrial maturation is fairly uniform in the different fundal sections (NOYES, 1956).

c) Vaginal Cytology (p. 534)

Daily preparation of a vaginal smear is a relatively simple and reliable method of roughly estimating the time of ovulation and assessing the state of ovarian function (RILEY, 1955). It is particularly suitable for the assessment of estrogen secretion during the follicular phase. It has been shown that during the follicular phase, the karyopyknotic index bears a significant relation to the logarithm of the total excretion of estrogens (JOHANNISON, 1961). In contrast to the situation in the endometrium, progesterone tends to have a nonspecific action on vaginal epithelium, and androgens, for example, can also produce the same effects. The vaginal smear at the time of ovulation has a characteristic "clean" appearance (p. 537 and Fig. 22). Results obtained are only reliable, however, when smears are taken continuously. Typical regressive changes occur in the individual cells within 1–2 days after ovulation (p. 537 and Fig. 23). These changes have been mentioned already and are not due to progesterone alone but can also be produced by a fall in the level of estrogens alone, by androgens or by corticosteroids.

Special practical importance is attached to exfoliative cytology when ovulation is stimulated by human gonadotropins and in the case of artificial insemination.

d) Cervix Factor (p. 538)

Examination of the cervical mucus, including assessment of the quantity, color, spinnbarkeit, crystallization (Fern test), penetrability to sperms (Sims-Huhner test) and the number of leukocytes contained, is another fairly simple method of determining the approximate time of ovulation and whether progesterone secretion has already started (Fig. 44). The width of the external os is also of practical use. There are also other methods of estimating the so-called cervix factor, such as measurement of the glucose content (BIRNBERG, 1958; DOYLE, 1959), the sodium chloride concentration, or the enzyme content (DONAYRE, 1965) or protein content (MOGHISSI, 1966). The cervix factor can also be assessed from its immunoelectrical behavior (HERVE, 1965). However, these additional methods are of no practical importance since they are unreliable and considerably more costly than assessment of the spinnbarkeit and the fern test.

e) Basal Body Temperature (BBT) (p. 546)

The simplest and most widely used method of estimating the time of ovulation and corpus luteum function is measurement of the temperature on wakening (VAN DE VELDE, 1905; VOLLMANN, 1942). The curve is based on the temperature-decreasing action of estrogens and the thermogenic effect of progesterone. It is believed that a plasma progesterone concentration of 25 ng/ml always results in a rise in BBT (ROSS, 1970). In order to obtain reliable results a number of conditions have to be observed (p. 624). In general, the temperature fall before the temperature rise is taken as the time of ovulation. Pregnancy occurring after one act of intercourse or a single artificial insemination has, however, shown that there is probably no strict relation between basal temperature and ovulation (ABARBANEL, 1958; HARTMANN, 1962). According to recent observations (JOHANSSON, 1972) a monophasic BBT is found in 12% of cycles even though other parameters investigated suggest that ovulation has occurred. It can therefore be supposed that the registration of a monophasic BBT during an otherwise seemingly biphasic menstrual cycle does not warrant the conclusion that the cycle is anovulatory.

f) Immunological Estimation of LH

The mid-cycle rise in LH secretion and excretion is taken as the immediate mechanism stimulating ovulation. Even biological assays of LH excretion have shown that increased LH secretion can already be determined before ovulation but that the peak of secretion is reached only after ovulation (FUKUSHIMA, 1964; MCARTHUR, 1958; ROSEMBERG, 1966). This has been confirmed by investigations with immunological estimations of LH (WIDE, 1962). Thus, it has become possible to determine the day on which ovulation will occur. The immunological method is simple and quick, but it is costly and not very precise.

Other tests for detecting ovulation, such as estimation of the action of oxytocin on myometrium contractibility (KNAUSS, 1935), pH changes in the vagina, electropotential changes between vagina and mons veneris (BURR, 1935)

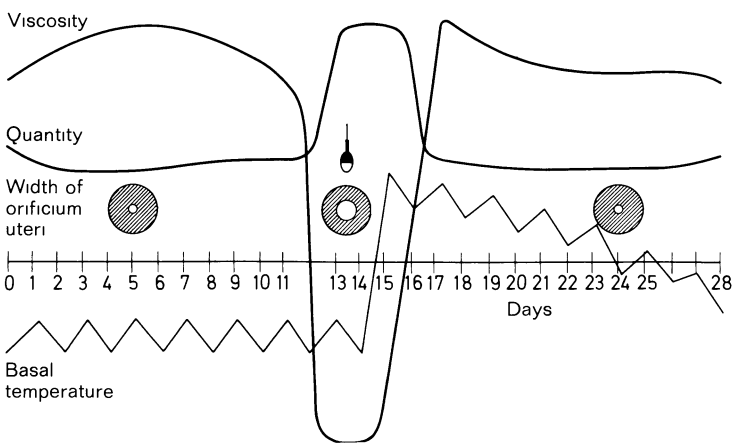


Fig. 44. Diagram showing the pattern of changes in mass and viscosity of the cervical mucus and the dilatation of the orificium uteri related to the basal temperature. (After BERNOTH, 1956)

composition of the blood and alveolar CO_2 tension (GOODLAND, 1952), have failed to gain practical importance for various reasons.

g) Clinical Symptoms

"Mittelschmerz" and mid-cycle bleeding (p. 608) are reliable signs of the time of ovulation, whereas intermenstrual discharge and changes in libido are of doubtful value.

h) Conclusions

Among the numerous methods available for detection of ovulation, the following investigations have proved to be quite reliable:

measurement of the basal body temperature;
endometrial biopsy;
pregnanediol excretion;
assessment of cervix factor;
vaginal cytology and immunological estimation of LH.

With the exception of the last method, none of the tests is reliable enough to be used as a single test. It is therefore necessary to use a combination of several (SÉGNY, 1933; BERGMANN, 1950; RAUSCHER, 1954, 1956, 1964; BELLER, 1962).

Stimulation of ovulation is discussed on p. 599, 630.

3. Inhibition of Ovulation

It has been deduced from the neuro-hormonal mechanism regulating the female cycle that the ovaries fail to function normally if the mid-cycle LH secretion can be successfully suppressed or if ovarian response to gonadotropins can be blocked.

Such a state of temporary physiological sterility occurs during pregnancy. This is a case of hormonal sterility, since the large amounts of estrogens and gestagens formed in the placenta almost completely suppress the secretion of pituitary gonadotropins.

HABERLANDT (1921) was the first to observe experimental hormonal sterility. He was able to show that ovaries of pregnant laboratory animals produced temporary sterility when transplanted into sexually mature female animals of the same species. Various investigators later showed (PAPANICOLAOU, 1926; PHILIPPS, 1937; MAKEPEACE, 1937) that progesterone inhibited estrus in rodents and produced temporary sterility in this way. BICKENBACH and PAULIKOVIVS (1944) found that intramuscular administration of 20 mg progesterone daily inhibits follicular maturation and ovulation in the female. Finally, Pincus and Chang investigated the mechanism of inhibition of ovulation in rabbit and rat by means of progesterone and its derivatives and metabolites. Oral progesterone has only a weak effect, and at least 300 mg must be taken orally every day to suppress ovulation in the woman (PINCUS, 1955). This prevents progesterone from being used as an ovulatory inhibitor in the woman.

In 1954, two groups of chemists reported the synthesis of 19-norsteroids (COLTON, ZAFFARONI). The progestive effect of these substances when given orally was described during the same year (HERTZ, 1954). Pincus and his team in particular deserve credit for examining the ovulostatic action of a large number of different steroids in rabbits and women (estrogens and other phenol steroids, androgens, progesterone derivatives and 19-nortestoids).

The first results on the contraceptive action of estrogen and progesterone mixtures in a large group of women appeared as long ago as 1957 (PINCUS, 1957; RICE-WRAY, 1957). From the large number of steroids so far investigated, various progestagens (p. 522, 546) and a few estrogens have proved to have an ovulostatic effect when given orally. The progestagens now in use as ovulatory inhibitors in the woman can be divided into two groups:

a) derivatives of 19-nortestosterone, the so-called 19-nortestoids (Fig. 45);

b) derivatives of 17α-acetoxy progesterone (Fig. 46).

Both groups were first tested by PINCUS and his team before being introduced therapeutically.

19-nortestoid is a chemical derivative of testosterone, the only difference between the compounds being the absence of the methyl group at C-19. Hence the name – 19-nor [no (r) = no methyl]. In addition, the 19-nortestoids now used as contraceptives have an ethinyl group at C-17, which stabilizes the molecule. These structural changes of the testosterone molecule result in a pronounced ovulostatic effect and the complete disappearance of androgenic action in the doses required for contraception.

Clinical investigations have shown that six 19-nortestoids are orally effective for contraceptive purposes in the woman (Fig. 45).

It has been shown that methylation and particularly chlorination at C 6 of 17α-acetoxy progesterone derivatives considerably increases the ovulostatic effect. Introduction of a double bond at C 5–6 also has this effect. The molecule becomes stabilized since breakdown of the progesterone molecule by hydroxylation at C 6 is impeded. All four derivatives of 17α-acetoxyprogesterone (Fig. 46) currently in use

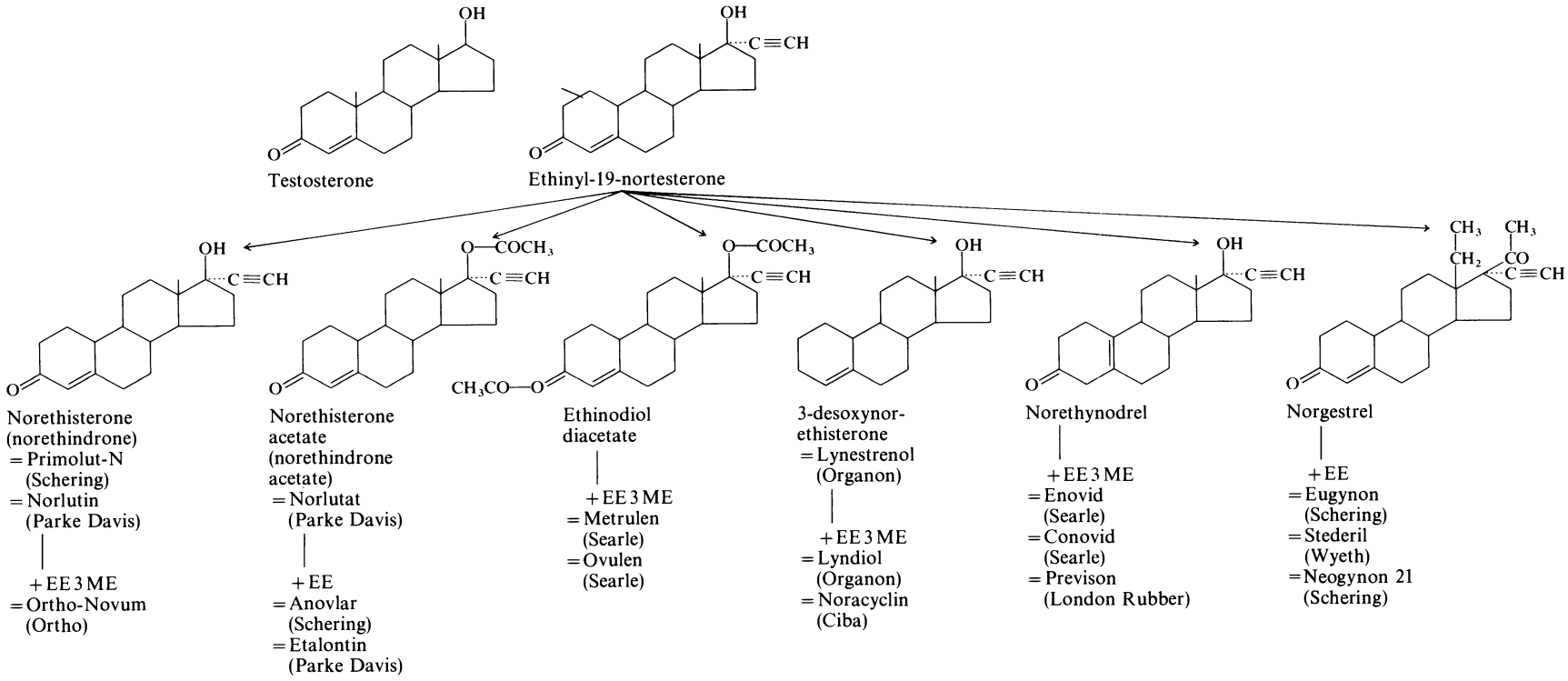


Fig. 45. Inhibitors of ovulation (19-nortestosterone derivatives) (SCHREINER, 1966)

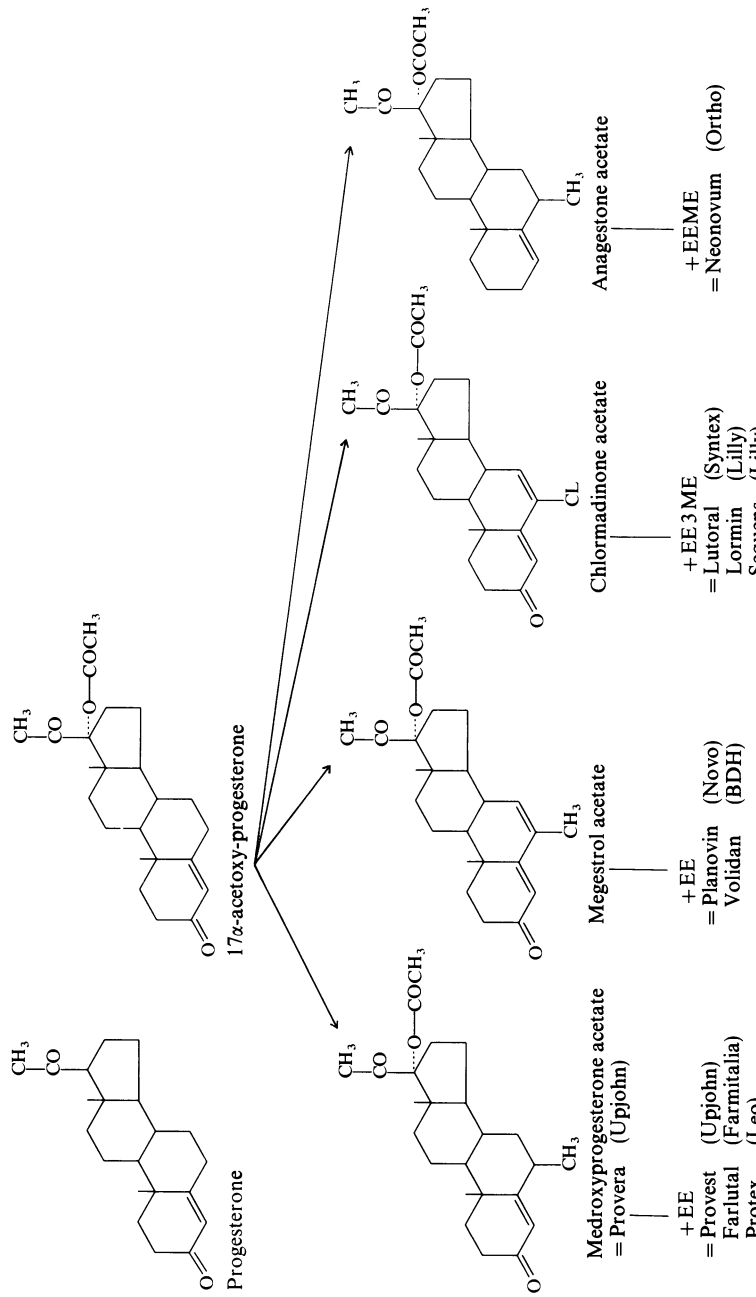


Fig. 46. Inhibitors of ovulation (17-acetoxy-progesterone derivatives) (SCHREINER, 1966)

for contraceptive purposes are therefore chlorinated or methylated at C 6. They are free of other hormone actions and cheaper to produce than 19-nortestoids. Since, unlike the nortestoids, they are not partly broken down into estrogens, the stabilizing effect on the endometrium is slighter. Breakthrough bleeding often occurs.

It is also known that estrogens and androgens in the correct doses can inhibit follicular growth (Fig. 46). The ovulostatic action of estrogens is now used in so-called sequential therapy (p. 573). Both groups of substances have significant disadvantages when used separately. Oral administration of estrogens often gives rise to gastrointestinal disturbances, painful swelling of the breasts, and severe breakthrough bleeding of long duration, due to endometrial hyperplasia. In addition, the onset of bleeding is uncertain. Androgenic substances in the doses necessary to suppress ovulation lead to virilization when administered for long periods.

All ovulostatic preparations contain 0.5–5 mg of a progestagen and 50–150 µg of an orally effective estrogen (Tables 33, 37). Either ethinyl estradiol (EE) or its 3-methyl ester (EE 3ME), the so-called mestranol, is used. When these substances are used, the dose of progestin can be reduced, since in the doses used they themselves have a central ovulostatic action. This also reduces the danger of unwanted side effects due to progestagens. The atrophic action of progestagens on the endometrium is reduced, so that breakthrough bleeding and amenorrhea seldom occur.

The contraceptive action of the estrogen-progestagen tablet is based primarily on inhibition of follicular maturation and ovulation. A hypothalamic inhibitory action is also involved, as well as a probably direct action on the ovaries (Fig. 47). The pre-ovulatory LH spurt (STEVENS, 1965) is suppressed due to inhibition of the LRH, and the response of ovarian enzymes to gonadotropins is also reduced (MATSUMOTO, 1960; LUNENFELD, 1963).

Table 33. Oral progestines and contraceptives. (Adapted from PINCUS, 1965)

	Progestagen		mg	Estrogen (µg)		Brand name
				EE3ME	EE	
19-Nortestosterone derivatives (estrane derivatives)	Δ^4-estrenolone	Norethisterone (norethindrone)	5	–	–	Primolut-N (Schering)
			5	–	–	Norlutin (Parke Davis)
			2	100	–	Ortho-Novum (Ortho)
		Norethisterone (norethindrone) acetate	5	–	–	Norlutate (Parke Davis)
			4	–	50	Anovlar 21 (Schering)
			2.5	–	50	Etalontin (Parke Davis)
		Ethynodiol diacetate	2	100	–	Metrulen (Searle)
			1	100	–	Ovulen (Searle)
			0.5	100	–	Ovulen (Searle)
		Norgestrel	0.5	–	50	Eugynon (Schering) Stediril (Wyeth)
	$\Delta^{5(10)}$-estrenolone	Norethynodrel	10	150	–	Enovid, Enavid (Searle)
			5	75	–	Enovid, Conovid (Searle)
			2.5	100	–	Enovid-E, Conovid-E (Searle) Previson (London Rubber)
	Estrenolone	3-desoxynorethisterone	5	–	–	Lynestrenol (Organon)
			5	150	–	Lyndiol (Organon) Noracyclin (Ciba)
			2.5	75	–	Lyndiol 2.5 (Organon) Noracyclin 22 (Ciba)
Progesterone derivatives (pregnane derivatives)	17-α-acetoxy-progesterone derivatives	Medroxyprogesterone acetate	2.5	–	–	Provera (Upjohn)
			10	–	–	Provera (Upjohn)
			10	–	50	Provest (Upjohn)
			5	–	50	Farlutal (Farmitalia)
			2	–	20	Protex (Leo)
		Megestrol acetate	4	–	50	Planovin (Novo) Volidan (BDH)
		Chlormadinone acetate	2	80	–	Lutoral (Syntex) Lormin (Lilly) Sequens (Lilly)
			3	100	–	Aconcen (Mark)
		Anagestone acetate	2	100	–	Neonovum (Ortho)

Reserpine and chlorpromazine also exert a similar inhibitory action on gonadotropin secretion via the hypothalamus (Fig. 47). Ovulation is successfully blocked but galactorrhea occurs due to simultaneous loss of prolactin inhibition (RATNER, 1964).

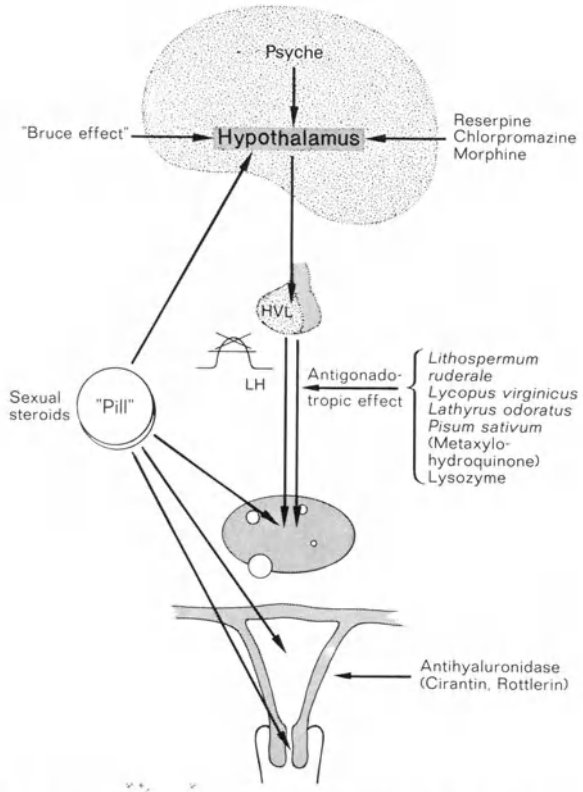


Fig. 47. Sites of action of various factors inhibiting fertility

The changes in the endometrium and cervical mucus caused by the progestagens also exert a contraceptive effect.

Chemical and physical changes occur in the cervical mucus. These changes are similar to those produced after ovulation due to the influence of gestagens and the cervical ascent of the sperms is impeded or made impossible (sperm barrier).

Fertility inhibition due entirely to corresponding changes in the cervical mucus is currently being intensively tested experimentally and clinically. This method has proved successful ("Minipill"). This represents considerable progress, since a contraceptive effect can be achieved with a minimal change in the reproductive system (p. 575).

The combination therapy (p. 573) also causes changes in the endometrium which impair blastocystic implantation (ROCK, 1956, 1957). Premature transformation with premature regression of the endometrial glands and endometrial atrophy arise after long-term treatment. These changes do not occur with the sequential therapy (p. 573) (Fig. 48).

During treatment with oral contraceptives, estrogen excretion in the urine is reduced, the values being lower than those at the time of ovulation or during the luteal phase (LORAINE, 1965; FLOWERS, 1966; WALLACH, 1968). Thus, there is a certain estrogen deficit during ovulostatic treatment. Prompt normalization of excretion values even after treatment lasting many years shows that this inhibition of estrogens is not irreversible (LORAINE, 1965; WALLACH, 1968).

During medication with estrogen-progestagen drugs, pregnanediol excretion is reduced to the values found during the follicular phase. However, different investigators have found pregnanediol values similar to those arising during the luteal phase (GOLDZIEHER, 1962, 1964; LIGGINS, 1967), but in the absence of pregnancy. Such values were found in 2.2 to 8.7% of the cycles produced with different compounds. The following explanations are under discussion:

1. formation of a corpus luteum during an "escape" ovulation, and
2. luteinization of the follicles.

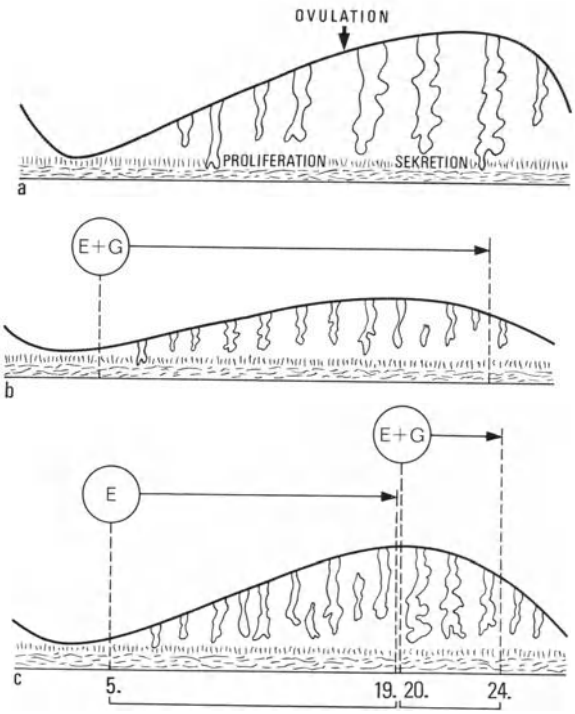


Fig. 48a–c. Diagram representing behavior of the endometrium a) in the natural biphasic cycle; b) during prolonged combined contraceptive treatment; and c) during sequential contraception (HALLER, 1968)

Table 34. Efficacy of various contraceptive methods. (Adapted from VENNING, 1965).

Method	Average number of pregnancies per 100 woman-years[a]
No contraception	115
Vaginal douching	31
"Safe period"	24
Vaginal jelly or cream	20
Coitus interruptus	18
Condom	14
Diaphragm (with or without jelly)	12
Intra-uterine contraceptive device	1–5.5
Ovulation inhibitors	0–3.1

[a] Pregnancies per 100 woman-years

$$= \frac{\text{No. of pregnancies}}{\text{No. of months in observation period}} \times 1200$$

The development of corpora lutea during conventional ovulostatic treatment has been reported (ERB, 1965).

Comparison with other contraceptive methods has shown that hormonal contraception is very effective (Table 34). The rate of pregnancy (= No. of pregnancies observed × 1200 divided by no. of observation months; Pearl Index), lies between 0 and 3, where failure is due partly to the fault of the patients and not to the drug. There are currently two methods of oral hormonal contraception in use:

Method I: combination therapy (PINCUS, 1955; ROCK, 1956)

Method II: biphasic or sequential therapy (GREENBLATT, 1961; GOLDZIEHER, 1963).

The original administration schedule introduced by Pincus (Pincus schedule) prescribes one estrogen-progestagen tablet daily from the 5th day for 20 to 22 days (combination tablet). The first day of the cycle is taken as the first day of menstruation. Withdrawal bleeding occurs 2–3 days after discontinuation of the tablets (i.e. on the 27th or 28th day of the cycle). Treatment is restarted on the 5th day after onset of bleeding. The following schedule is simpler: 3 weeks intake, 1 week tablet-free; when 21 tablets are used, treatment is always started on the same day of the week and there is a free interval of 7 days. If 22 tablets are used, the first and last tablets are taken on the same day of the week, and the free interval is only 6 days.

Sequential therapy is also started on the 5th day of the cycle. Pure estrogen tablets (80–100 µg EEME) are taken for the first 14–15 days, followed by conventional combination tablets for the next 5–7 days. The dose of estrogens selected is adequate to suppress ovulation, and progestagens are used only to achieve secretory transformation and limited withdrawal bleeding. The sequential therapy is considered to be less safe than the combination method, since it is based entirely on inhibiting ovulation. It does, however, have the advantages that endometrial atrophy with the associated danger of amenorrhea are removed, and that unwanted side effects are less common. The withdrawal bleeding is more severe.

With both forms of therapy, observation of two conditions is necessary to achieve a definite anti-fertile effect:

1. medication must be started before the 6th day of the cycle, and

2. the tablets must be taken regularly.

Corresponding investigations (MATSUMOTO, 1960) have shown that follicular maturation and ovulation can no longer be effectively suppressed if medication is started after the 5th day of the cycle. Steroids are quickly metabolized, so that a single dose exerts a reliable inhibitory action on the ovary for only about 36 hours. If a tablet is forgotten in the evening, it should be taken no later than the following morning. If the time interval is longer, it is pointless to double the dose. In order to maintain the intake schema, the patient should continue with the usual daily intake until the 24th day of the cycle. When a tablet has been omitted a conventional contraceptive method must be used in addition to the ovulation inhibitor for the remainder of the cycle. It has been shown statistically that the number of failures or pregnancies is related to the number of tablets omitted (PINCUS, 1965). The fact that an ovulostatic is only as reliable as the patient taking it is one of the main reasons why the failure rate is so high in the places where contraception is most needed, namely in the developing countries. Whereas in Holland, only 6% of women stop taking the tablets with in one year (SWAAB, 1964), 28% of women in Singapore (SAN, 1963) and 74% in Madras (MENON, 1964), stop within one year, and in Puerto Rico 82 to 92% no longer take the tablets for contraception after $2\frac{1}{2}$ years (PINCUS, 1965).

Doctors are often asked how quickly the contraceptive action develops after the beginning of tablet intake. Studies in a large number of women have shown that 6.3 of every 1000 ovulations occur during the first 7 days of the cycle (RICHTER, 1963). Premature ovulation occurs almost without exception in women with short cycles. In these uncommon cases, tablet intake started on the 5th day is too late. To ensure full protection it is therefore wise to advise the couple to use another conventional contraceptive method during the first

10 days of the first intake cycle, or to tell the patient to start taking the tablets from the first day of the cycle. This is only necessary during the first cycle.

To what age must contraception be practiced? Although the likelihood of conception decreases rapidly after the 30th year and is only 3% at 40 (MÜNZER, 1934), a few pregnancies have occurred at 51, and despite the increasing frequency of abortion, have been carried to term (WYLER, 1957). One third of all pregnancies in women of 45 or over end in miscarriages (JAVERT, 1957).

Table 35. Treatment with ovulation inhibitors (SCHREINER, 1966). (Available on prescription only)

Before commencement of a treatment

Case history, Assessment of general condition } see contraindications

Examination of genital organs including breasts: colposcopy, cytotest after PAPANICOLAOU, bimanual palpation.

During treatment

First check-up after 2 months, then annual check-ups, annual cytotest after PAPANICOLAOU.

Indications

a) Contraception, polymenorrhea, oligomenorrhea, hypermenorrhea, dysmenorrhea, premenstrual syndrome, functional sterility

b) Functional sterility, genital hypoplasia, endometriosis

c) Manipulation of menstruation

Treatment schedule

ad a) Cyclic treatment: 1 tablet daily on days 5–24 (25) of cycle.

ad b) Continuous treatment: 1st week 1 tablet daily, 2nd and 3nd weeks 2 tablets daily, increase daily dose by 1 tablet every 2 weeks to a maximum of 4–6 tablets daily as long-term treatment.
Duration in functional sterility 3–4 months, in genital hypoplasia 3–6 months, in endometriosis 9 months or longer.

ad c) 1 tablet daily for 4 days before the expected menstrual period. If time of period is to be changed by more than 1 week, increase dose to 2 or more tablets daily after 7 days.

Contraindications

Carcinoma of the reproductive organs including the breast. Tendency to thromboembolism: status varicosis, severe obesity, recent surgery or delivery. After cerebrovascular trauma; cholestasis, e.g. after jaundice in pregnancy. Pregnancy (nortestoid preparations).

To be used with caution during lactation, in the presence of uterine fibromyoma or tendency to water retention: cardiac insufficiency, bronchial asthma, migraine, epilepsy; diabetes mellitus.

No other substance has been so extensively examined for undesirable side effects as the estrogen-progestagen mixture in the "pill". The severity and type of the side effects are not only dependent on the dose of the single hormone fractions but also on psychological factors to a particular degree (PINCUS, 1965). About every 5th patient complains of side effects (HALLER, 1968). These may take the form of bleeding disturbances, or may be of a generalized nature (PINCUS, 1965; HALLER, 1968). The frequency usually decreases after the tablets have been taken for 2–3 months.

No form of organic damage or disturbances in the ova or regulation has been detected even after contraceptive tablets have been taken for years. There is no carcinogenic action (Table 36) though the growth of a hormone-dependent carcinoma (carcinoma of the breast and body of the uterus) already present can be accelerated by the estrogen components (Table 35). The only real risk associated with ovulostatic treatment, is the increased danger of thromboembolism (Royal College of General Practitioners) (VESSELS, 1968; INMAN, 1968). According to statistical investigations, the risk of a non-fatal lung embolus is 10 times higher (VESSELS, 1968). The risk of a fatal lung embolus or cerebral venous thrombosis is in any case very slight, but it is 7 times higher when ovulostatics are taken (INMAN, 1968). This risk, however, is no higher than during pregnancy and the danger is significantly less than the general risks encountered in pregnancy.

In addition to the unwanted side effects, ovulostatics also demonstrate a series of desirable secondary effects, so that the indications for use are not restricted to contraception. A number of gynecological disorders can be treated (Table 35):

Table 36. Incidence of cancer during long-term estrogen substitution (LAURITZEN, 1968)

Author	Patient-years	No. of patients	Duration of treatment (years)	Calculated incidence	Observed incidence
GORDON (1961)	1200	120	14	12–15	0
WILSON (1962)	2604	304	17	20	0
WALLACH and HENNEMAN (1959)	1480	292	25	22	5
SCHLEYER-SAUNDERS (1960)	–	500	15	30	0
GEIST (1941)	–	206	5.5	12	0
Total	–	1422	–	96	5

endometriosis (p. 553, 633);
dysfunctional bleeding: polymenorrhea, oligomenorrhea, hypermenorrhea, secondary amenorrhea (p. 585, 603);
functional dysmenorrhea, "mittelschmerz" (p. 618);
postponement of menstruation;
functional sterility (p. 623);
genital hypoplasia.

Contraindications. Carcinomas of the reproductory organs including the breasts;
tendency to thromboembolism: state after thromboembolism, varicosis, excessive obesity, after surgery or postpartum (6 weeks), after cerebovascular accidents;
during lactation;
cholostasis (not, however, liver cirrhosis or chronic hepatitis).
Caution is indicated in:
cardiac failure, bronchial asthma, migraine, epilepsy because of tendence to retain water;
diabetes mellitus (glucose tolerance is reduced by ovulostatics).

Since contraceptive tablets are not all the same and they are contraindicated in certain instances they are only available on prescription; thorough medical examination is essential before and during the treatment (Table 35).

In the last few years, the practical value of the contraceptive action of long-term treatment with small oral doses of progestagens (so-called "luteal supplementation"), has been examined (RUDEL, 1965; MARTINEZ-MANAUTOU, 1966) and proved effective.

This form of treatment is of special interest, since apart from its simplicity, it produces antifertile effects exclusively by altering the cervical mucus and the endometrium, hypothalamic-pituitary-ovarian function remaining intact. Experience obtained in the use of the so-called "minipill" in a daily dose of e.g. 500 mg chlormadinone acetate has shown that the rate of pregnancy is 3.7 per 100 women years, which is only slightly higher than that for other methods in use, although 70% of the women concerned ovulated. Disadvantages are frequent breakthrough bleeding (20% in the first cycle) and a tendency to oligomenorrhea (about 20% of the trial women showed a cycle lasting between 36 and 59 days).

GREENBLATT (1967) has developed a tablet which only has to be taken once every month. It has therefore been described as the "one-pill-a-month contraceptive". It contains an oral estrogen with a long-lasting effect (the 3 cyclopenthyl ester of ethinyl-estradiol 2–5 mg) and a short-acting progestagen, (chlormadinone acetate 5–8 mg). The tablet is taken on day 25 of the cycle. Ovulation inhibition throughout the month is achieved by the constant estrogen level. The investigations performed so far on its reliability have yielded a result of 1.5

Table 37. Hormonal contraceptives (inhibitors of ovulation)

Preparation (manufacturer)	Composition (mg)	No. of tablets
19-nortestosterone derivatives		
Anovlar 21 (Schering)	4 norethisterone acetate 0.05 EE	21
Enovid (Searle)	2.5 norethynodrel 0.1 EEME	20
Etalontin (Parke Davis) Norlestrin (Parke Davis)	2.5 norethisterone acetate 0.05 EE	20
Eugynon (Schering) Steridil (Wyeth)	0.5 norgestrel 0.05 EE	21
Gynovlar (Schering)	3 norethisterone acetate 0.05 EE	21
Lyndiol (Organon) Noracyclin (Ciba)	5 lynestrenol 0.15 EEME	20
Lyndiol 2.5 (Organon) Noracyclin 22	2.5 lynestrenol 0.075 EEME	22
Orlest (Parke Davis)	1 norethisterone acetate 0.05 EE	21
Ortho-Novum (Ortho)	2 norethisterone 0.01 EEME	21
Ovulen (Searle)	1 ethynodiol diacetate 0.1 EEME	21
17-hydroxyprogesterone derivatives		
Aconcen (Merck)	3 chlormadinone acetate 0.1 EEME	21
Estirona (Lilly)	15 tablets each containing 0.08 EEME 5 tablets each containing 2 chlormadinone acetate and 0.08 EEME	20
Menoquens (Novo)	16 tablets each containing 0.1 EE 5 tablets each containing 1 megestrol acetate and 0.1 EE	21
Planovin (Novo) Volidan (B.D.H.)	4 megestrol acetate 0.05 EE	21
Sequens 21 (Lilly)	14 tablets each containing 0.08 EEME 7 tablets each containing 2 chlormadinone acetate and 0.08 EEME	21
Cyclo-Farlutal (Farmitalia)	5 medroxyprogesterone acetate 0.075 EE	21

EE = ethinyl estradiol;
EEME = ethinyl estradiol methyl ether = mestranol.

pregnancies to 100 women years. This corresponds to that of the sequential therapy, and its chances of being accepted appear to be good.

The same effect is produced by intramuscular administration of an estrogen-progestagen preparation (the so-called "one-shot-a-month"), on the 8th day of the cycle (RICE-WRAY, 1965). A depot estrogen e.g. estradiol-valerianate is combined with a depot gestagen (16α,17α-dihydroxy-progesterone acetophenide). Dependence on the doctor, and frequent breakthrough bleeding are disadvantages of this method. Sometimes the bleeding is so severe that it necessitates a curettage.

According to experience gained so far the injection of 150 mg medroxy-progesterone-acetate every three months or 300 mg every 6 months can also prevent pregnancy with a high degree of certainty (TAYMOR, 1964). As with the combination tablet, a multi-locular anti-fertile effect is involved. Ovulation inhibition, atrophy of the endometrium and gestagenic alteration of the cervical mucus occur. Severe disadvantages are the recurrent breakthrough bleeding and amenorrhea, which may last for several months.

Finally, investigations with depot progestagens are currently in progress. Silicone tablets are injected into the tissue, and micro-doses are released through the capsule wall. Conception can possibly be prevented for a desired time by refilling the capsule. This method is still in the preliminary stages, so that nothing definite can be said about the dosage, effectiveness and side effects.

For the sake of completion, the so-called "morning-after pill" and "month-after pill" must be mentioned here. Neither is an ovulation inhibitor. The so-called "morning- or day-after pill" (MORRIS, 1966), consists of a large dose of estrogen (50 mg diethyl-stilbestrol or 2–5 mg ethinyl-estradiol daily) and is given for the first 5 days after possible conception. Presumably implantation is inhibited, and possibly tubal transport is also accelerated so that the ovum reaches the endometrium at a stage where it is still too immature for implantation. Failures are usually due to too low a dosage of estrogens.

Anti-metabolites such as 6-mercaptopurine or 5-fluorouracil can interfere with the development of a pregnancy during the first four months (month-after pill) (MORRIS, 1967). There is probably an inhibitory action on blastocystic mitosis.

There is a second group of substances which inhibit synthesis of progesterone so that withdrawal bleeding and expulsion of the fetus result, but clinical trials in Sweden have failed. The so-called "Bruce effect" (BRUCE, 1960; PARKES, 1961) must also be mentioned (Fig. 47). This effect is based on the observation that 80–90% of female mice do not become pregnant when they are brought into close contact with strange male mice or their urine shortly after copulation. LTH production is probably inhibited via the hypothalamus by a strong transient odorous substance.

F. Transition Periods in the Woman

1. Puberty and Menarche

(see also Chap. XIX, p. 1034)

In the life of a woman different stages of development can be differentiated: infancy, puberty, adolescence, sexual maturity, climacteric and senility (Fig. 49).

The boundaries of the different phases of life are indistinct and somewhat arbitrary.

Puberty begins with the development of the pubis (STUART, 1946). Pre-puberty is often termed as the time between the onset of the development of the breasts and that of pubic hair. The mechanism causing the development of puberty is still not clear. Hypothalamic maturity (see below) and normal thyroid and adrenocortical function seem to be essential, whereas the state of maturation of the hypophysis and ovaries (p. 554) is irrelevant (ABRAMS, 1967). The importance of the pineal body is not clear (Chap. IV, p. 73). From studies made in animal experiments, it is assumed that the inhibitory center in the anterior hypothalamus continuously loses its sensitivity to the sexual steroids, resulting in a decrease of its inhibitory effect on the eminentia mediana, which in turn causes the liberation of gonadotropin-releasing hormone already present (VAN DER WERFF TEN BOSCH, 1966). The increased androgen formation is probably responsible for the puberal spurt of growth. At this age (adrenarche) androgens are mainly produced in the rapidly growing zona reticularis of the adrenal cortex and perhaps also in the hilus cells of the ovaries. At the same time, the pelvis and breasts develop under the influence of the androgens. It is possible that androgens also have some important effect on maturation of the hypothalamic "sexual center", since maturation of this center is a decisive factor in the onset of vegetative ovarian function, the so-called *oophorarche*. Due to gonadotropic stimulation, estrogens are formed in the ovaries, and estrogen excretion in the urine soon exceeds 10 μg per 24 hours. The influence of estrogens brings about development of the primary and secondary sexual characteristics (p. 532ff. for the biological action of estrogens). So for example, after the

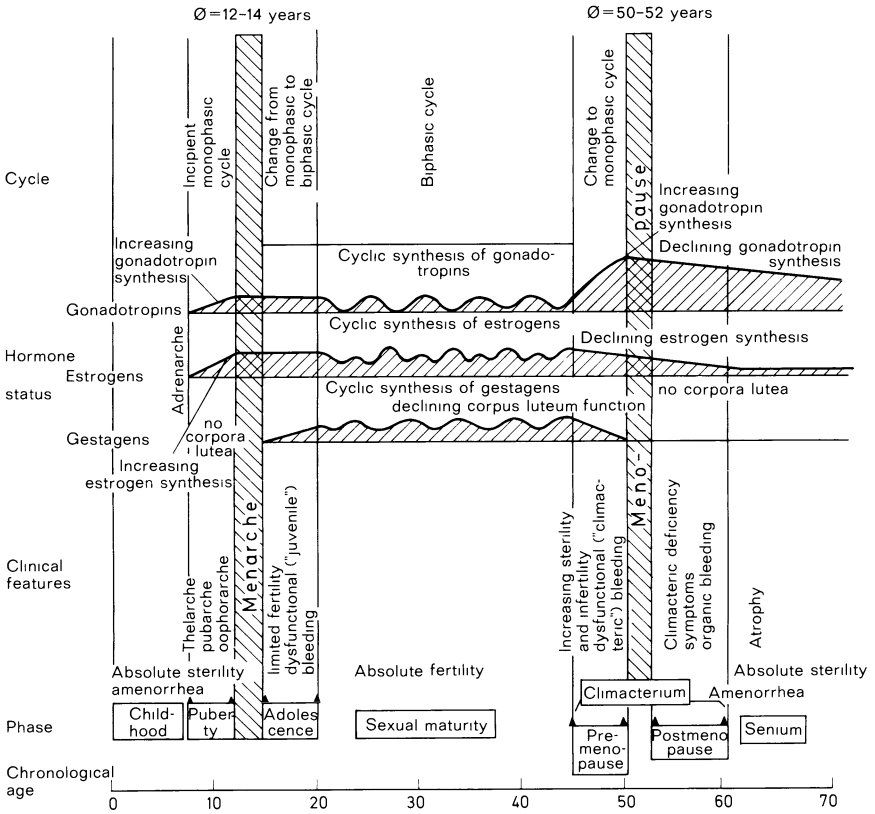


Fig. 49. Phases in the life of woman

10th year, the uterus rapidly increases in size (Fig. 26) and the original ratio of body to cervix of 1 : 2 is reversed (p. 538).

However, the ovaries can react to gonadotropic stimulation to form sexual steroids long before the oophorarche (PASCHKIS, 1955; ABRAMS, 1967). No gonadotropins are formed before the 5th year (BETTENDORF, 1962) and excretion begins only 3–4 years before menarche (JOHNSON, 1959). Hypophyses of juvenile animals implanted into hypophysectomized adult animals are capable of functioning fully as soon as the hypophyseal portal circulation has been re-established (HARRIS, 1952). This is proof that hypothalamic maturity is essential for the onset of formation of pituitary gonadotropins.

The influence of the androgens is predominantly responsible for the development of the pubic hair and causes the so-called *pubarche* and the formation of axillary hair shortly before menarche.

Androgens, somatotropin and estrogens in particular lead to the development of the puberal breasts, the so-called *thelarche*. The breasts, however, only undergo complete development under the additional influence of gestagens.

The most striking event at this time is *menarche*, which is an obvious manifestation of incipient cyclic ovarian function. This marks the onset of *adolescence* which lasts until regular biphasic ovarian cycles are established i.e. until the end of growth. The peak velocity of increase in height ends almost without exception with the onset of menarche (DEMING, 1957). Growth in height usually ends 3 years after menarche. The inhibiting influence of the increased estrogen secretion on height is indicated by the fact that an early menarche is frequently associated with a small, pyknic stature, and a later menarche with a large leptosomatic type. The average age at which menarche occurs varies from population to population. In Europe and North America it is currently between 12 and 14 years (Fig. 50), menarche arising between 11.8 and 16 years in 90% (GRIMM, 1948), and between 11 and 16 years in over 95% (STAEMMLER, 1964). When menarche occurs between 16 and 18 years, since these ages are taken to be outside normal limits (SOUTHAM, 1966) it is justifiable to speak of a *delayed menarche*. Menarche arises this late in less than 5% of young women (GRIMM, 1948). After completion of the 18th year, the

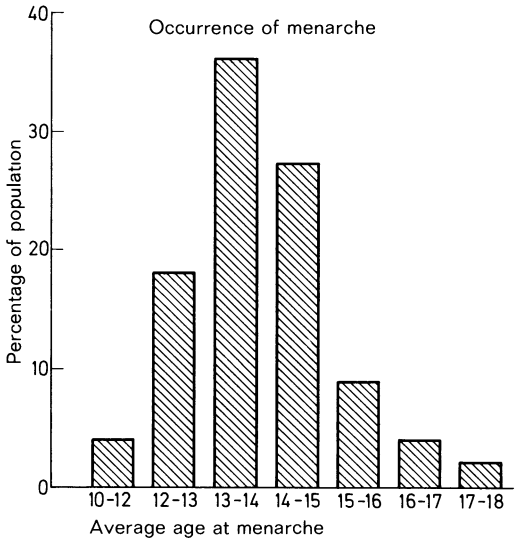


Fig. 50. Age at menarche in American girls. (After WELLENBACH, 1967)

first period arises spontaneously in only 0.3% of these women (GRIMM, 1948). The term *primary amenorrhea* is used when menstruation has failed to occur after completion of the 18th year. Precocious puberty is spoken of when menarche arises before the 9th year. The age at which menarche occurs is primarily genetically determined, and is almost always the same for mother and daughters and sisters (TANNER, 1962) and practically identical in monozygotic twins (PETRI, 1935). Distribution of the age at which menarche arises within a population group gives a gaussian binominal curve corresponding to genetic determination (Fig. 50). The onset of menarche is also influenced by environmental factors. Socio-economic factors in particular, and nutritional conditions (protein intake, vitamins) play a part as well. Menarche usually arises a few months earlier in the higher social classes than in girls of lower social level (TANNER, 1962). This is also illustrated by the fact that menarche arises earlier in urban than in rural populations. The influence of nutrition is particularly apparent in chronic nutritional disorders such as diabetes mellitus and ulcerative colitis. Menarche was also later during the war years. One of the most convincing arguments for the influence of nutrition is the observation made in Lapland. The average age at menarche was found to be constant at 16.5 years between 1870 and 1930 in the sections of the population which continued their nomadic life, whereas the age at menarche declined by two years during the same period in the neighboring settled farmers (TANNER, 1968). The higher incidence of disease and the greater exposure to stress situations in poorer population groups have an additional retarding effect on menarche (RENOLDS, 1947; MANDL, 1952; VARON, 1963). In contrast to results obtained with animals, light seems to exert an inhibiting influence on menarche in women; it is usually earlier in blind girls (ZACHARIAS, 1964). Climate and race seem to influence menarche much less than nutritional conditions (HENTON, 1958; LEE, 1963). Menarche occurs at the same age in Nigerian girls belonging to higher tribes and in Eskimo girls (ELLIS, 1950; LEVINE, 1953) whereas the first menstruation occurred $1\frac{1}{2}$ years earlier in Japanese girls born and brought up in California than in Japanese girls born in California but brought up in Japan (ITO, 1942). In these cases, nutritional conditions are probably the decisive factor again. On the other hand, in ancient days, menarche appeared to arise a year later in German girls than in Roman girls. This difference between south and north Europeans can still be observed (BACKMAN, 1948). The average age at menarche fluctuated considerably during the course of the centuries. It occurred at the age of 12 in Hindus 2500 years ago (BENERJEE, 1961), at 14 in Europe during the period of classical antiquity, and at 18 at the end of the 18th century (BACKMAN, 1948). Since then there has been a continuous decline of about 4 months per decade in age at menarche (PORTMANN, 1967) (Fig. 51). Increased stress to the individual due to urbanization (BENNHOLDT-THOMSON'S urbanization trauma, 1942), and various inherited factors are the basis of this mainly Western phenomenon (LENZ, 1965).

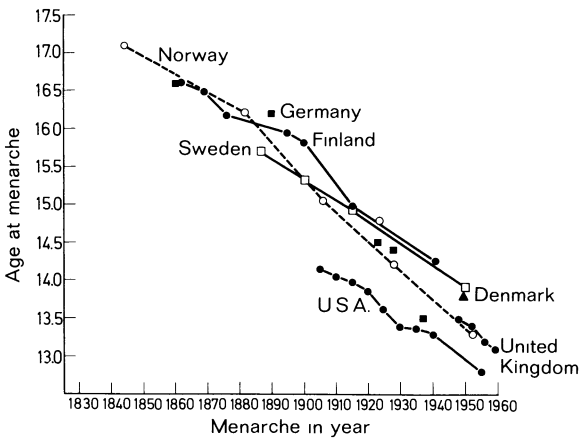


Fig. 51. The secular shift in age at menarche between 1830 and 1960. (After TANNER, 1962)

Certain prognostic conclusions about later sexual function can be deduced from the age of menarche. Labile or irregular cycles with

dysfunctional bleeding and sterility arise more frequently after a late or retarded menarche ($14\frac{1}{2}$–16 years) (p. 603) (STAEMMLER, 1964; SOUTHAM, 1966). The cause of delayed menarche or primary amenorrhea can be situated at one of the three functional levels: hypothalamus and hypophysis, ovary and thyroid or adrenal cortex, or uterus and vagina (see primary amenorrhea, p. 585ff.). In most cases only variations of the normal are present. Investigation of delayed menarche is similar to that of primary amenorrhea (p. 594ff.) (SOUTHAM, 1966).

2. Climacteric and Menopause

a) Definition

The terms "climacteric", "menopause", and "postclimacteric" are used differently in different countries and often appear as synonyms. In the following discussion, we use only the terminology recommended by the Nomenclature Committee of FIGO (Féderation internationale de gynécologie et d'obstétrique) (KAISER, 1956). This terminology is factually and etymologically correct (Fig. 49).

The *menopause* is the last menstruation or in retrospect, the time of the last menstrual period before one complete year of amenorrhea (TIETZE, 1952; BREWER, 1954; KAISER, 1965 etc.). Climacteric (the change of life) depicts the transition period from the time of full sexual maturity to the point at which ovarian function is lost. It includes a few years before and after the menopause and is followed by senility. The years prior to the menopause are termed as premenopause, and the years thereafter up to senility as postmenopause (WENNER, 1959; BÉCLÈRE, 1963).

The climacteric or premenopause begins about 2 to 3 years before the menopause with a resultant fall in estrogens and a rise in gonadotropins. The postmenopause ends when estrogen excretion reaches the values found in senility. This phase lasts on average 6–8 years after the menopause (KAISER, 1965).

An *early menopause* (precocious climacteric) is spoken of when the last menstruation occurs before the 40th year of life. The term *late menopause* (climacterium tarda) indicates that the last period did not occur until after completion of the 54th year. The incidence of early menopause has been variously quoted as 1.2% (GOECKE, 1959) and 8% (KEETEL, 1964). The menopause is late in 6.9% of women (GOECKE, 1959).

Like puberty and adolescence, the climacteric represents a normal, physiological transition period between sexual maturity and senility. It is not an illness in itself but the development of deficiency symptoms which may become pathologic may cause some women to look upon it as an illness (p. 582ff.). The climacteric is peculiar to the human so that it is impossible to investigate any associated processes in animals.

b) Age at Menopause

As the age at menarche has been declining, so climacteric and menopause have been occurring progressively later in the past 130 years. The proportions of these changes are about the same for menarche and menopause, and up to 1950 were approx. 3 years per 100 calendar years (BACKMAN, 1948).

Until the Middle Ages, the average age at menopause was around 40, while between 1500 and 1840 it was 45 years. Since then, there has been a rapid and progressive rise in the age at the onset of the menopause, so that in 1940 the average age at menopause was 48 years (BACKMAN, 1948). This continuous rise in the age at which menopause occurs shows a pronounced rhythm, with a phase duration of about 20 years (Fig. 52). Since 1940 there has been another acute rise. Whereas menopause occurred at the age of 49.5 (HAUSER, 1961) or 49.3 (DÖRING, 1959) in 1960, it was found that it occurred at 50.1 years (FROMMER, 1964) or $52\frac{2}{12}$ years (DE WAARD, 1964) in 1964. Menopause occurs between 45 and 55 years in about 50% of women (GOECKE, 1959) (Fig. 53). The tendency for menopause to arise later is supported by the decreased incidence of early menopause and the increased numbers of women with late menopause.

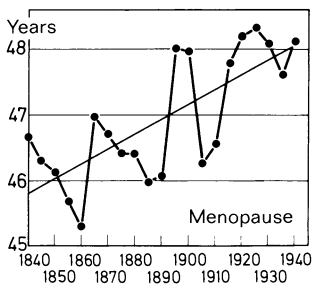


Fig. 52. Changes in mean age at menopause per decade and for every fifth year in Europe, 1840–1940. (After BACKMAN, 1948)

The long-term trend in the ages at which menarche and menopause arise, which is statistically significant, also appears to be valid for individual women. There is a connection between the age at menopause and that at menarche, in the sense that an early menarche generally

means that menopause will be late, and conversely a late menarche indicates an early menopause (BACKMAN, 1948; GOECKE, 1959). The duration of sexual maturity in the woman has increased during the course of the last 100 years (Fig. 54). This is also applicable to the average life expectancy. A few investigators doubt that there is any connection between duration of sexual maturity and life expectancy (SCHAEFFER, 1906, 1908; BENJAMIN, 1960).

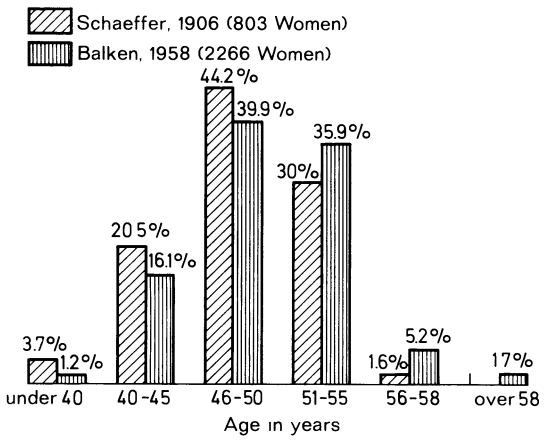


Fig. 53. Range of age at menopause. (After GOECKE, 1959)

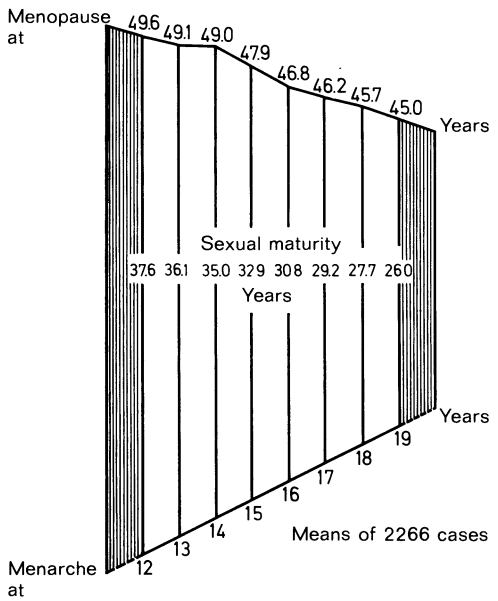


Fig. 54. Temporal dependence of the menopause on the menarche. (Balken in: GOECKE, 1969)

c) Causes of the Onset of Menopause

Present-day knowledge suggests that the cause of the onset of the climacteric and menopause lies within the ovary itself. It is not primarily due to the absence of primordial follicles ("burnt-out ovaries"), since a small number of these follicles is present even after the menopause (BLOCK, 1952) (p. 517) but results rather from the reduced response to gonadotropic stimulation. It is probable that the cause of this is not in the oocyte, but in the ovarian vascular system, which undergoes senile changes earlier than other vessels elsewhere in the body (SOHMA, 1908). The increased gonadotropin levels may accelerate this process, since administration of high doses of gonadotropins to animals has been observed to lead to sclerosis of the ovarian vessels (LAURITZEN, 1968). The ovary is the only endocrine gland which begins to decrease in weight from the 30th year (Fig. 26). The ovary may temporarily increase in size during the premenopause due to follicular persistence. In the postmenopause there is increasing shrinkage with fibrous tissue formation. The cortex in particular becomes narrower due to the disappearance of germinal parenchyma. Cortical granulomas consisting of large, polygonal cells with lipoid deposits arise in the hilus (HERTIG, 1944); they are similar to thecal cells, but may produce androgens (p. 527). Hilar vessels degenerate (SOHMA, 1908). The surface of the ovary becomes grooved like the brain, and becomes whitish-yellow in color. The weight of the ovary can decline to half or one third of its weight during sexual maturity (WATZKA, 1957).

Estrogen formation decreases due to the reduced response of the follicular apparatus to gonadotropic stimulation. FSH release is reduced to a lesser extent, and LH liberation is increased (p. 561). This causes the formation of tertiary follicles, but there is no terminal spurt of growth due to the disturbed feedback mechanism. Estimations of estrogen excretion have shown that the ovaries can be stimulated by the administration of exogenous gonadotropins in the presence of elevated HPG formation even 1–2 years (PAULSEN, 1958) or 3–5 years (depending on the investigator) (LAURITZEN, 1968) after the menopause and that a few ovulations can even occur spontaneously in the postmenopause, as is confirmed by occasional pregnancies arising at this phase (SHARMAN, 1962). Studies in monozygotic twins have shown that hereditary factors are of considerable importance in determining the age at menopause. In monozygotic twins, the average difference in age at menopause is two months, as against 19 months in dizygotic twins (GOECKE, 1959). The familial occurrence of climacterium praecox and tarda confirms the influence of genetic factors (MAYER, 1928). In addition, as in the menarche, environmental influences, particularly nutritional factors (MCBRIDGE, 1956) have some

effect on menopause. Wasting diseases and unknown endogenous influences also affect the organism so that menopause occurs later. This is also true of women with fibroids and carcinoma of the body of the uterus.

d) Hormonal Conditions

The first result of reduced response of the follicle to gonadotropins is a decrease in the amount of estrogens produced. A fall in estrogen excretion can sometimes be demonstrated even between the 40th and 45th year (KAISER, 1965), but in these cases there is no further significant fall during the premenopause (Table 38). Other investigators, however (FURUHJELM, 1966), have found no correlation between age and estrogen excretion between 40 and 50 years. We and a few other authors have observed that not infrequently the estrogen excretion is temporarily considerably increased during the premenopause (ZONDEK, 1947) while gonadotropin excretion is found to be increased at the same time (PAPANICOLAOU, 1969) (so-called polyfolliculine stage after ZONDEK, 1947). It appears that in these cases, the central "feeler system" no longer responds to the elevated estrogen titer (p. 557). If estrogen excretion is less than 7–10 µg/24 h for a long time no more bleeding occurs because endometrial stimulation is inadequate (BROWN, 1959). Estrogen excretion falls steadily during the postmenopause, and values between 5–18 µg/24 h have been reported (DICZFALUSY, 1961; PAPANICOLAOU, 1969). The senile level of 5µg/24 hours is attained after 7 years at the earliest. Excretion values of over 20 µg/24 h in late postmenopause and senility indicate a pathologic process (DICZFALUSY, 1961). It is interesting to note that cyclic variations in estrogen excretion may arise immediately after the menopause (BULBROOK, 1957) without bleeding. The reduced estrogen secretion during the climacteric is reflected in the *vaginal cytology smear*: the pyknosis index falls to an average value of 10% during the postmenopause (DICZFALUSY, 1961). In a few cases, the pyknosis index is as high as 30% even 30 years after the menopause in the absence of any kind of estrogen administration (SMOLKA, 1956; PUNDEL, 1957). One must, however, remember that apart from sexual steroids, nonhormonal substances such as digitalis, senna, cascara and tetracyclines can also exert a proliferative stimulus on vaginal epithelium. Intensive proliferation in the vaginal epithelium can be caused only by estrogens, and has been observed in 9.1% of women after the menopause, exceptionally as long as 14 years after the menopause (KAISER, 1965). It is probable that ovarian

Table 38. Excretion of gonadotropins and estrogens in women after the 41st year (KAISER, 1965)

Age	Gonadotropins in mg HMG 20 A/24 h	Total estrogens in µg/24 h
Normal cycles	13 ± 7 (12)[a] (maxima)	30.1 ± 6.7 (17)[b] (average)
41–45	30 ± 40 (12)	22.3 ± 5.9 (10)
46–50	53 ± 42 (13)	20.7 ± 14.0 (31)
51–55	82 ± 57 (10)	12.7 ± 8.7 (10)
56–60	63 ± 54 (6)	7.2 ± 2.0 (5)
61–65		6.8 ± 2.5 (6)
66–75	41 ± 30 (12)	5.9 ± 1.8 (5)

[a] No. of cycles examined.
[b] No. of cases.

estrogens are steadily replaced by adrenal estrogens in the urine to the same extent as germinal parenchyma disappears, so that the estrogens originate mainly from the adrenals during late postmenopause and in senility (residual estrogens) (DICZFALUSY, 1961). The estrogen excretion does not change after surgical castration during a normally occurring menopause (BULBROOK, 1958), whereas estrogen values in the urine fall to unmeasurable levels following adrenalectomy in the postmenopause (BULBROOK, 1957). On the other hand, vegetative symptoms due to loss are often intensified after ovariectomy during the postmenopause (HELLER, 1944). This may be due to the effect of castration in the early postmenopause when the basal estrogen secretion of the ovarian stroma still has a certain quantitative effect. It is also of interest that a slight estrogen excretion can still persist during the postmenopause even after ovariectomy and adrenalectomy (BULBROOK, 1957); its origin is uncertain. Incubation studies have shown that the ovaries produce mainly androgens during the postmenopause (PLOTZ, 1967; MATTINGLY, 1969), so that the ovarian stroma in aging ovaries functions as an endocrine gland (ENGLE, 1955; RICE, 1966).

During the premenopause the fall in estrogens leads to a diminished arrest of the hypothalamo-hypophyseal system, which leads in turn to a rapid rise in the amounts of FSH and LH excreted. The rise in LH excretion is somewhat less than that in FSH excretion. Particularly high values arise during the premenopause (Table 27, p. 561). These values lie between 40–400 IU/24 h for FSH and between 15–90 IU/24 h for LH where the total gonadotropin excretion is 3–40 mg Eq/24 h (KELLER, 1970). KAISER (1965) found a raised gonadotropin excretion even after the 40th year, but this observation has not been confirmed by other investigators (JOHNSON, 1959; KELLER, 1970).

During the postmenopause there is a steady decrease and any type of cycle is lost (Fig. 49). The variations in FSH and LH excretion during this phase of the postmenopause are not so great in the individual case (KELLER, 1968) as have been reported by some authors (ALBERT, 1966).

Even in senility, the gonadotropin excretion is many times higher than during sexual maturity (JOHNSON, 1959; KAISER, 1965). JOHNSON therefore speaks of the "eternal youth" of the pituitary. In contrast, 17-ketosteroid excretion in the woman decreases from the 20th year onward, there being a significant negative correlation between age and the quantities excreted (FURUHJELM, 1966). There is no "adrenopause", however.

e) Clinical Features

α) Premenopause

Progressive generative ovarian insufficiency arises during this phase of life. Corpus luteal function is deficient and cycles are often anovulatory. Clinically, bleeding disorders characteristic to the premenopause frequently arise. Only a little over a quarter (23.5%) of women enter the postmenopause with no cyclic disturbances. Disorders in rhythm (44.7%), and type (26.8%) are observed in 71.5% of cases (GOECKE, 1959). Twin studies have shown that hereditary factors play a considerable part in determining this course of events (MORETTI, 1953). Dysfunctional bleeding termed as "climacteric bleeding" is the predominant feature at this stage. It arises about 3 years before the onset of menopause (Fig. 55) with increasing frequency and especially often in the last year. It constitutes 70–80% of bleeding disorders during the premenopause, in the presence of an intact ostium uteri (KAISER, 1965). The characteristic climacteric bleeding is continuous, arises after a prolonged interval, and is associated with glandular-cystic hyperplasia due to follicular persistence (p. 607). The diagnosis "dysfunctional bleeding" should, however, be made only after organic causes have been excluded. This applies in particular to carcinomas of the cervix and of the body of the uterus, which, like polyps and fibroids, increase in incidence at this age (Fig. 55). Dysfunctional bleeding presents predominantly as disturbances in the bleeding rhythm.

Disorders in the type of bleeding, particularly metrorrhagia, are much more frequently caused by organic factors.

Functional sterility arising from progressive insufficiency of the corpus luteum, is of only little practical significance at this stage of life. The likelihood of pregnancy at 30 is thought to be 30% that at 20, only 11% at 35 years and 3% at the age of 40 (MÜNZER, 1934). Pregnancy can, however, occur up to the 51st year (WYLER, 1959), but every third pregnancy in women over 45 ends in abortion (JAVERT, 1957).

β) Postmenopause

Estrogen production finally falls below the physiological threshold (hypofolliculine stage) and is no longer adequate to stimulate endometrial bleeding. The postmenopause is reached at this stage. The amounts of estrogens produced are, however, still sufficient to cause significant proliferation of the endometrium. Dense endometrial proliferation, which has been described during the postmenopause, is probably due to delayed maturation of an ovum, which occasionally occurs. In so-called cystic atrophy, a few silent glandular tubes become cystically dilated. Ovulation can exceptionally arise several years after menopause, as has confirmed by the occurrence of pregnancies during the postmenopause (SHARMAN, 1962).

The clinical picture of the postmenopause is characterized by bleeding disorders, and by various types of psychological and somatic disturbances. Bleeding at this stage after 6 months of amenorrhea is dysfunctional in almost 50% of cases (KAISER, 1965; BÄTTIG, 1968). Later, organic causes are of primary importance, particularly carcinoma of the body of the uterus. Only about 20% of all types of bleeding at this phase of life are dysfunctional (Fig. 55), whereas 30–50% are due to malignant tumors (BÄTTIG, 1968; GOECKE, 1959).

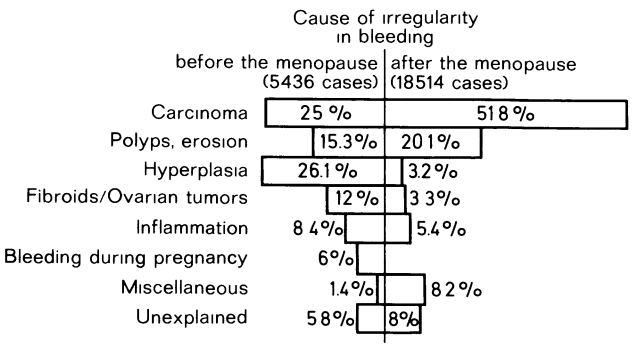


Fig. 55. Frequency of various causes of bleeding in the climacteric. (After HARBORT in GOECKE, 1959)

The climacteric syndrome (menopause syndrome), which includes vegetative (so-called climacteric vegetative syndrome) and psychological disorders during the climacteric, is the

presenting feature in the majority of cases. The data about type and frequency of disorders vary widely between 2% (MARANON, 1929) and 85% (ISRAEL, 1967) according to investigator and population group. There seems to have been an increase during the last decade. The climacteric features must be evaluated as a true illness in a quarter to a third of these women, and treatment is then necessary (BOSHANN, 1966; ISRAEL, 1967; LAURITZEN, 1968). The causal factor is the fall of estrogens. Castration leads to symptoms similar to those arising with the natural menopause (Fig. 56); substitution therapy with estrogens removes these symptoms. The level of the gonadotropin excretion is not, however, related to the severity of the symptoms. The poor adaptation of the organism to the fall in estrogens leads to diencephalic false regulation with vegetative dysfunction. A state of neurovegetative equilibrium usually arises after a few years, but sometimes only during senility. Disturbance of the functional circle consisting of diencephalon, hypophysis and ovary can result in false regulation of other functional circles, and can lead to hyperthyroidism, or less frequently hypothyroidism.

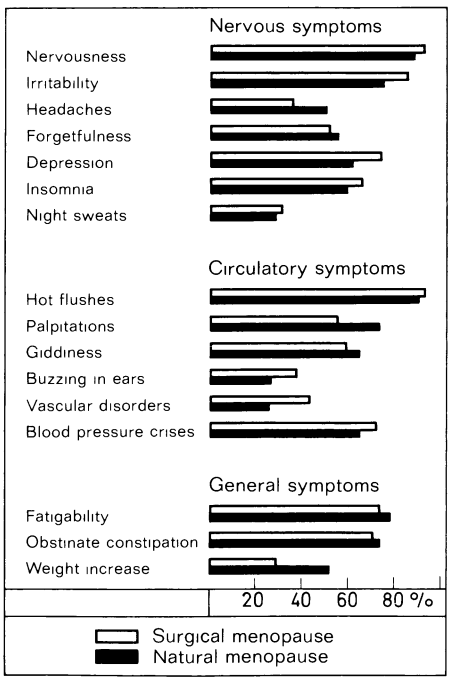


Fig. 56. Postmenopausal symptoms. (After LAURITZEN, 1968)

The climacteric symptoms are numerous (Fig. 56). Duration and severity vary with the individual, both being dependent on constitution and to a lesser extent on external environmental conditions. The woman "experiences the form of climacteric appropriate to her vegetative and psychological constitution" (WIESEL, 1924). Any woman who is already vegetatively and psychologically labile is predisposed to symptoms due to loss of function. Climacteric dysfunctional symptoms can precede the menopause by some years and usually disappear within a few years, although they do exceptionally persist into advanced age. The pathogenesis is not explained. It has never been shown that increased amounts of serotonin, adrenaline, noradrenaline or histamine are liberated. Hot flushes are the main clinical feature of the vegetative disturbances. As a rule these flushes are followed by an outbreak of sweating and this is probably the only symptom which is pathognomonic to the climacteric since it never arises otherwise. These hot flushes are ergotropic sympathetic attacks with hyperemia in the region supplied by the cervical sympathetic nerves (HAUSER, 1961), i.e. in the face, neck, thorax and hands, in those areas where erythema pudendum also arises. Often there is also a rise in blood pressure (HOFF, 1959). In diagnosis and treatment of the climacteric syndrome, it must be remembered that similar symptoms can also be due to organic cardiovascular changes.

Psychological disturbances during the climacteric are not a direct result of the endocrine adjustment, but are rather psychological reactions to the changed position in life. A conscious or unconscious fear of the loss of femininity moves into the foreground with the emotional lability. This fear is based on real changes: loss of the ability to bear children, decreased sexual capacity, loss of female charms, independence of the children, displacement at work by younger female colleagues etc. (DEUTSCH, 1948). The intelligent woman with a healthy psychological understanding usually disposes of these psychological factors without developing psychological disorders. Constitution, upbringing and particularly the situation in life have a considerable influence on the type of reaction.

Disorders due to organic factors arise more frequently after the menopause. Thus there is an increase in the incidence both of genital diseases such as kraurosis and pruritus vulvae, senile colpitis, urethro-cystitis, carcinomas of the fundus of the uterus, ovaries and breast, and of extragenital diseases; unstable blood pressure, hypertension, rise in cholesterol and lipoproteins in the blood, obesity, coronary sclerosis, myocardial infarction, osteoporosis and osteoarthrosis all increase in frequency. Some of these conditions are due directly to the reduction in estrogen production (dys-

pareunia, senile colpitis, urethro-cystitis). There is probably a causal relation between the loss of estrogens and osteoporosis and the syndrome of hypertension, obesity, coronary sclerosis and myocardial infarction (WEGELIN, 1944; HOFF, 1959; BERKSON, 1964; PRILL, 1966; THOMAS, 1966; PLOTZ, 1967; DAVIS, 1967; PSCHYREMBEL, 1968). The loss of ovarian function does predispose women to obesity (matron's fat, old woman's fat) (HOFF, 1959). The climacteric accounts in some way for 37% of cases of obesity in women (climacteric obesity) (SCHULZ, 1958). In castrated women there is an increase in weight of over 10 kg in 18% (TRIASSAC, 1956), 5% put on over 20 kg (BAHNER, 1955); after a natural menopause 22 to 39% of women put on weight (BARRET, 1933, 1931; WIRTZ, 1935). Large-scale statistical studies from American life insurances also show that "climacteric obesity" does exist. Metabolic changes, reduced physical activity and elevated calorie intake (worry fat) are causal factors. Constitutional factors are also of importance.

Senile osteoporosis in the woman is discussed in Chap. XIV (p. 867). This type of osteoporosis is about 5 times more common in the female than in the male (REIFENSTEIN, 1958).

f) Therapy

In 1900 the life expectancy of American and European women was 51 years. By 1960, it had already risen to 74 years (MENDUKE, 1967). This means that ovarian function is now absent for one third of a woman's life. Since the testes continue to function to an advanced age, there is no analogous phase of life in the male and no parallel in the animal kingdom. The question of a general continuous substitution during the postmenopause and senium is therefore under discussion. The following facts have now been established: although the theoretic advantages of general prophylaxis with estrogens cannot be disputed, until now there are no certain bases for assessing the value of long-term estrogen treatment, and the duration of treatment with estrogens such as is now being employed empirically during the postmenopause is still too short to permit any conclusions (PLOTZ, 1967).

Substitution treatment is indicated in cases where the symptoms due to loss of estrogens take the form of a true illness. Substitution is absolutely contraindicated in cases of carcinoma of the reproductive system, the breast included, and for five years following treatment of these cases. Caution is indicated in the presence of uterine fibroids, endometriosis, mastopathia cystica and particularly with any cholostatic hepatopathy, since 17-alkalized steroids promote stasis (p. 575). In general the doses prescribed are too high, particularly immediately after the menopause, so that iatrogenic bleeding occurs in about 10% of patients under treatment (LAURITZEN, 1968). The optimal dose is the lowest possible dose which leads to the disappearance of symptoms due to loss of estrogens. Treatment must be adjusted to the individual, and oral treatment is especially well suited for substitution. Yearly examination by a specialist is essential for women receiving substitution treatment. Metrorrhagia calls for investigation by a fractionated curettage. The cytotest is only 65% accurate in cases of carcinoma of the corpus uteri and is not adequate for investigation of these cases of metrorrhagia (HELD, 1955).

Assessment of the onset of action, the duration of action and the therapeutic value of a drug depend mainly on observations of the climacteric symptoms before and after treatment. Estimation of the so-called "climacteric index" has proved to be of practical value (KUPPERMAN, 1959). Double-blind trials against placebo are essential. The "Kupperman index" is a simple arbitrary system, in which 10 recognized climacteric symptoms are given a number from 1–4. The intensity of the symptoms is also given such a numeric factor (Code: symptom not present = 0, mild = 1, average = 2, pronounced = 3) (Table 39).

Table 39. Assessment of the severity of climacteric symptoms or the therapeutic value of a preparation in the climacteric index (KUPPERMAN, 1959)

Symptoms	Factor	Intensity	Numeric conversion
Vasomotor symptoms	4	M (2)	8
Paresthesia	2	+ (3)	6
Sleeplessness	2	+ (3)	6
Nervousness	2	M (2)	4
Depression	1	+ (3)	3
Giddiness	1	L (1)	1
Weakness (fatigue)	1	L (1)	1
Arthralgia and myalgia	1	M (2)	2
Headache	1	L (1)	1
Palpitations	1	+ (3)	3
Climacteric index (sum)			35

[a] 0 = no symptom = 0; L = mild = 1; M = moderate = 2; + = severe = 3.
[b] Numeric conversion = factor * intensity.

Multiplication of the symptom factor by the intensity factor gives the "numerical conversion". The "climacteric index" is obtained by adding the results for each of the 10 symptoms together. A "climacteric index" of 35 or more indicates severe climacteric dysfunctional symp-

toms. An index between 20 and 25 indicates an average case, whereas values of about 15 indicate mild cases. The "climacteric index" should fall to 15 or less if treatment is successful.

Good results are achieved with conjugated estrogens (p. 550). The risk of iatrogenic bleeding is reduced since their action on the endometrium is rather slight ("soft" estrogens). The following doses are usual:

Presomen, Oestrofeminal or Premarin, 1.25 mg daily for 3 weeks, followed by a break of one week.

Estradiol preparations can be used successfully instead of conjugated estrogens, e.g. Progynova (estradiol valerianate) 1 mg daily for 3 weeks, then a break of one week. In some cases a combined preparation of conjugated estrogens with a tranquilizer has the most favorable effects when psychological disorders are predominant: Menrium (conjugated estrogens with Valium) 3 × 1 tablet daily for 3 weeks, then a week's break, followed by 1–2 tablets daily for 3 weeks and a week's break and so on.

During the first two years after the menopause, there is an increased risk of hemorrhage during treatment with estrogens. On account of this, some authors recommend biphasic treatment with one of the modern oral contraceptives. We think the value of this is debatable, since the risk of breakthrough bleeding is merely replaced by the risk of withdrawal bleeding. Both types of bleeding indicate a curettage.

Higher doses of estrogens can of course be prescribed for hysterectomized women when indicated.

G. Ovarian Dysfunction

1. Amenorrhea

Menarche occurs between the 10th and 16th year in about 95% of all women in European countries (GRIMM, 1948; STAEMMLER, 1964; WELLENBACH, 1967), and it only arises spontaneously in approximately 0.3% of cases after completion of the 18th year (GRIMM, 1948). It is therefore reasonable to speak of a *primary amenorrhea* when menarche has failed to occur even after completion of the 18th year in a person of female phenotype.

The point at which secondary amenorrhea may be diagnosed is still somewhat arbitrary. According to most authors, secondary amenorrhea is present when three or more months have elapsed since the onset of the last menstrual period in a patient who previously menstruated more or less regularly. A pregnancy must of course be excluded (ROCK, 1945; BICKERS, 1948; JONES, 1954). Secondary amenorrhea may occur physiologically during lactation, in the post menarche and in the postmenopause. The physiological forms of amenorrhea, which also include amenorrhea during pregnancy, must be distinguished from pathologic amenorrhea.

In addition, *generative* amenorrhea (amenorrhea of the 1st stage) must be distinguished from *vegetative* amenorrhea (amenorrhea of the 2nd stage). In the first type, generative ovarian function is absent i.e. there is no follicular rupture and no corpus luteum is formed, while in the second type no estrogens are produced either. Classification into hypergonadotropic amenorrhea (where excretion of pituitary gonadotropins (HPG) is increased) and hypogonadotropic amenorrhea (where HPG excretion is reduced) is justified for prognostic reasons. Hypergonadotropic amenorrhea has a considerably worse prognosis.

Amenorrhea is merely a symptom and not a disease. The causes of the symptom amenorrhea are extremely varied and the connections are often complex and still partly unexplained. This fact is reflected in the large number of recommended classification principles. A classification system considering the individual links of the functional cycle: hypothalamus, hypophysis, ovary, and uterus, and based on the etiology and pathology, is of practical use for didactic, diagnostic, and therapeutic reasons (Table 40). Some arbitrary classifications are inevitable. Thus classification of the Stein-Leventhal syndrome under ovarian amenorrhea is at least debatable. This pluriglandular disorder could be classified with as much justification under amenorrhea due to hypothalamo-pituitary factors. This is also applicable to symptomatic ovarian insufficiency in extragenital endocrine disorders, which is classified under hypothalamic forms of amenorrhea.

a) Primary Amenorrhea (Table 41)

In the majority of these cases amenorrhea is due to congenital ovarian defects, often genetic in nature (gonadal dysgenesia, ovarian hypoplasia) or to congenital malformations (Mayer-Rokitansky-Küster syndrome, gynatresia). It is rarer for it to be due to pseudohermaphroditism (testicular feminization, congenital and prepubertal adrenogenital syndrome). Pathologic chromosomal idiograms are therefore often encountered (PHILIP, 1965; JACOBS, 1961). The pathogenesis is more frequently ovarian than uterine. Primary amenorrhea due to hypothalamo-hypophyseal failure or to dysfunction

Table 40. Classification schedule for amenorrhea

1. *Hypothalamic amenorrhea*

a) Organic	Inflammation (meningoencephalitis) Degeneration (Laurence-Moon-Biedl syndrome) Neoplasm Trauma
b) Psychogenic (exogenous, endogenous)	Psychoreactive (emotional amenorrhea) Anorexia nervosa (mentalis) Pseudocyesis (grossesse nerveuse, grossesse imaginaire) Genuine psychosis Simple, postpartum ovarian insufficiency Chiari-Frommel syndrome (sometimes Forbes-Albright syndrome)
c) Iatrogenic	Sexual steroids, especially progestagens, phenothiazines, reserpine
d) Nutritional	Chronic malnutrition
e) Symptomatic in dysfunction of correlating organs	
Adenohypophysis	M. Cushing
Ovary	Endocrine-active ovarian tumoras or non-neoplastic ovarian cyst
Adrenal cortex	Adrenogenital syndrome M. Addison
Thyroid	Hypo- or hyperthyreosis
Pancreas	Juvenile diabetes mellitus
f) Idiopathic (dysfunctional)	

2. *Pituitary amenorrhea (adenohypophyseal)*

a) Organic	M. Simmonds: Inflammation (TB, syphylis, actinomycosis, giant-cell granulomas, etc.) M. Simmonds: Degeneration: postpartum ischemic necrosis = M. Sheehan (Sheehan's syndrome) Neoplasm (adenoma, craniopharyngioma) Forbes-Albright syndrome = Ahumada-del Castillo syndrome = Argonz-del Castillo syndrome (chromophobic adenoma) Acromegaly (eosinophilic adenoma) Trauma
b) Idiopathic	

3. *Ovarian amenorrhea*

Gonadal dysgenesis (or agenesis)
Chromosome idiogram 45/XO, 46/XY, 46/XX and mosaic)
(Swyer's, Rössle's or Turner's syndrome)
Ovarian hypoplasia
Ovarian dysfunction; polycystic ovaries or Stein-Leventhal syndrome
Destruction of ovarian tissue (tumors, inflammation)

Ovarian hypofunction, e.g. physiologic amenorrhea post partum and post abortum, autonomic ovarian insufficiency, so-called burnt-out ovary in post menopause and old age)

4. *Uterine amenorrhea*

Mayer-Rokikansky-Küster syndrome (uterus bipartitus cum vagina solida)
Traumatic amenorrhea, uterine synechiae (*Asherman's* syndrome)
Métrose de réceptivité (Moricard's syndrome)
Silent menstruation

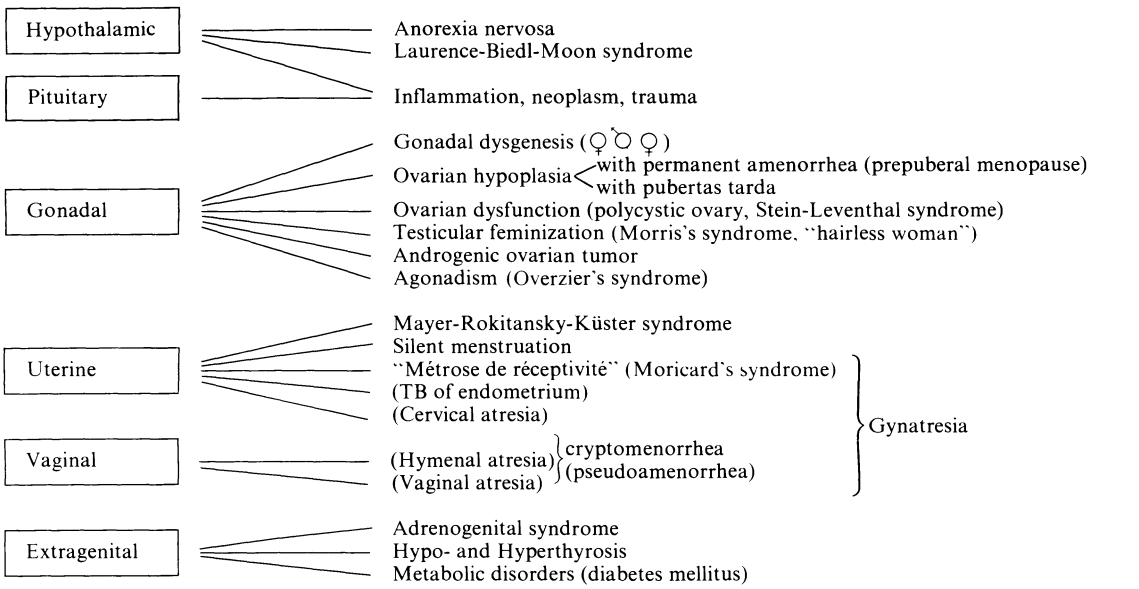
5. *Amenorrhea in hermaphroditismus verus and pseudohermaphroditismus masculinus with external feminization*

(testicular feminization)

Addendum

False amenorrhea in gynatresia (hymenal, vaginal, or cervical atresia)

Table 41. Primary amenorrhea: classification of primary amenorrhea by site and pathogenic aspects



of extragenital incretory organs and metabolic disorders is common (Fig. 57) (KUMSCHIK, 1966; PHILIP, 1965).

Patients with *ovarian primary amenorrhea* usually show hypogonadism. Genitalia and breasts are underdeveloped, pubic and axillary hair sparse. This type of amenorrhea occurs in individuals with gonadal dysgenesia and ovarian hypoplasia.

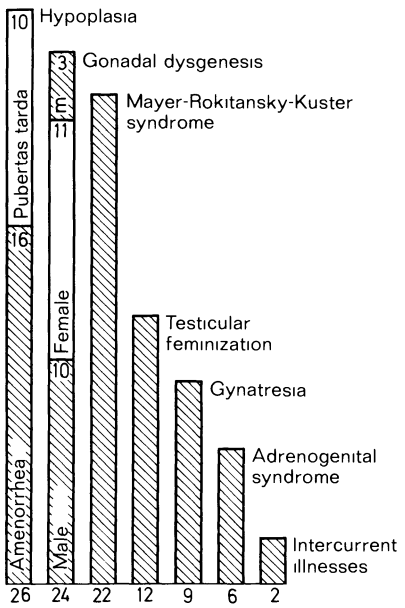


Fig. 57. Distribution of 101 cases of primary amenorrhea by type of disorder. (After KUMSCHIK, 1966)

In *gonadal dysgenesia*, there is a congenital disorder of the gonadal anlage due to a defect of the sex chromosomes. This has been discussed in detail in another section (PRADER, p. 713). In these cases, there is a gonadal rudiment with complete absence of the germinal cells. It is usually the malformations or the small stature and not the amenorrhea which cause the patient to consult the doctor, particularly the pediatrician. Primary amenorrhea with hypogonadism can be the first symptoms leading to further investigation in a case of gonadal dysgenesia where growth is normal (so-called Swyer's syndrome).

So-called *ovarian hypoplasia* is another quite common cause of primary amenorrhea (STAEMMLER, 1964). The etiology and phenology are very irregular in this group of patients, disorders of the menstrual cycle in the presence of hypoplasia, but typically formed or cylindrically formed ovaries being the only common features. The proportion of germinal parenchyma may be reduced (primary hypoplasia = *hypogenesia*) or may be abundant in comparison to the sparse ovarian stroma (secondary hypoplasia). Both forms of hypoplasia represent an anomaly of the anlage, probably due to a genetic defect (PASCHKIS, 1955). This signifies a developmental anomaly (Table 42). It is quite possible that a congenital defect in the ovarian enzyme system is present (STAEMMLER, 1963). Accelerated follicular atresia due to an unknown hypergonadotropic phase is under discussion (BÉCLÈRE,

Table 42

Ovarian hypoplasia

(After STAEMMLER, 1964)

Primary (hypogenesis)		*Secondary*
Deficient germinal parenchyma defective anlage?		Germinal parenchyma relatively abundant Underdevelopment
	Forms	
Basic form: small, cylindrical ovary	Marginal form: small, but typically-formed ovary	Basic form: small, cylindrical ovary
Bordering on *gonadal dysgenesis*		Bordering on *normal*
	Clinical picture	
Tall or short stature (45%)		Noncharacteristic
Underdeveloped primary and secondary sexual characteristics		Primary sexual characteristics hypoplastic, and secondary ones noncharacteristic
Possibility of prepuberal menopause		Possibility of pubertas tarda
HPG elevated (hypergonadotropic hypogonadism)		HPG often lowered (hypogonadotropic hypogonadism)

1963). It is probable that some of the disorders are of hypothalamo-hypophyseal nature, and still undetectable. Primary amenorrhea with primary hypoplastic ovaries is also described as *pre-pubertal menopause*. Secondary hypoplastic ovaries can lead to the clinical picture of *delayed puberty* (pubertas tarda).

Growth disorders in the form of exaggerated (>170 cm) or diminished growth (<155 cm) have been observed in almost 50% of all women with primary ovarian hypoplasia. Genital organs, breasts, pubic and axillary hair are poorly developed due to the primary estrogen deficiency. Elevated HPG activity in the urine is characteristic (hypergonadotropic hypogonadism) (KINCH, 1965). In contrast to this, there is no uniform phenotype for women with secondary ovarian hypoplasia, since the hypoplastic genital organs may be associated with infantile or normally developed secondary sexual characteristics. The urinary excretion of pituitary gonadotropins is often reduced (hypogonadotropic hypogonadism).

Primary amenorrhea may exceptionally arise in the syndrome of *polycystic ovaries* (p. 609).

Agonadism (Overzier syndrome) is rare. The external genitalia are of female type, but only the vaginal opening is present, the vagina itself being absent. Primitive Müller's and Wolffian ducts are the only internal genitalia present. Overzier's first two cases were siblings (OVERZIER, 1961). All patients so far described have had male chromosomes (XY).

Patients with a malformed uterus or without a uterus constitute a second large group with primary amenorrhea.

Inhibition malformations of the distal part of Müller's duct are quite common in the so-called *Mayer-Rokitansky-Küster-syndrome* (MRK syndrome) (bipartite solid uterus without vagina) (HAUSER, 1961). We observed this syndrome 12 times within 4 years. Other authors (KUMSCHIK, 1966) have reported that this syndrome accounted for as many as 22% of their cases of primary amenorrhea. The uterus usually consists of a solid muscular cord divided in two. Both uterine cornua with lateral club-like processes are immediately next to the ovaries, and extend medially in the form of a comma. They are connected by a retrovesical peritoneal structure (ligamentum latum). The malformation increases in the craniocaudal direction (Fig. 58). Genetically, a developmental inhibition of the distal section of the Müller's filaments during the second month of embryonal life, gives rise to the MRK syndrome, the causal genesis is unknown. These subjects have a 46/XX chromosomal configuration. Inheritance must be excluded on the basis of the cases reported so far.

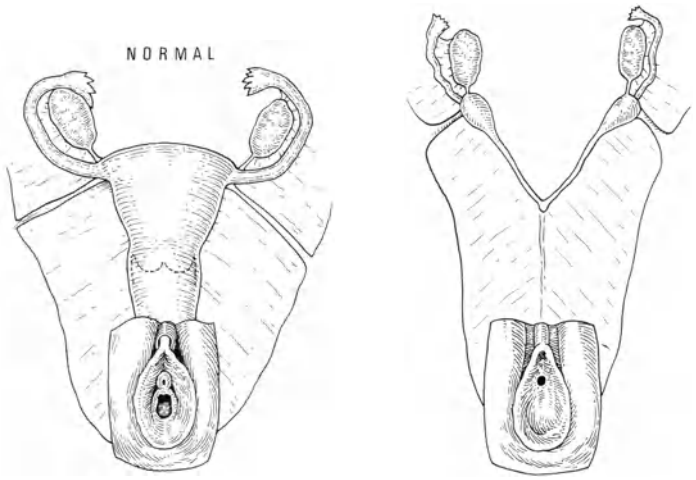


Fig. 58. Diagram showing normal female reproductive tract contrasted with Mayer-Rokitansky-Küster syndrome: no vagina, divided uterus, a single thread in the middle and club-shaped at the sides. The ovaries are placed higher than normal. (After HAUSER, 1961)

Symptoms: Primary amenorrhea and the inability to have intercourse are the most frequent reasons for consulting the doctor. The patients only rarely seek medical advice because of primary sterility.

Very rarely, primary amenorrhea is due to an *imperforate hymen*, *congenital vaginal closure* in the cranial third, or *closure of the cervix* (MCKUSICK, 1964). These defects lead to hematocolpos, or hematometra and hematosalpinx (false amenorrhea). The cramp recurring monthly in the lower abdomen, increasing in severity and radiating to the lumbar region, together with the demonstration of a retrohymenal, or uterine or parauterine (tubal) painful tumor rapidly leads to the right diagnosis.

Endometrial TB exceptionally leads to primary amenorrhea. The ovarian cycle is maintained in these patients. This can be shown by the biphasic basal body temperature and the colpocytogram. The estrogen test is negative.

Patients with primary amenorrhea very occasionally have normal ovarian and endometrial cycles and normal fertility (ISRAEL, 1967). Absence of cyclic withdrawal bleeding is attributed to the absence of the hypothetic uterine hemorrhagic factor (silent menstruation or amenorrhea spuria).

Testicular feminization must be mentioned as another form of primary amenorrhea (so-called Morris syndrome, "hairless woman") (MORRIS, 1953). This form of intersexuality has been described fully in another section (p. 726).

In contrast to secondary amenorrhea, primary amenorrhea is seldom due to functional disorders of the hypothalamo-hypophyseal system resulting from infections, circulatory disturbances (infarct, hemorrhage, internal hydrocephalus) tumors (neoplasms, aneurysms of the internal carotid artery), malnutrition or psychological factors. Organic changes in the region of the pituitary and hypothalamus always lead to primary amenorrhea together with disorders of growth and development as well as impaired metabolism, water balance and sleep.

Craniopharyngiomas affecting the hypothalamus are discussed on p. 120.

Primary amenorrhea due to *psychological* factors (BENEDEK, 1952; MICHEL-WOLFROMM, 1963; CONDRAU, 1967) arises in the absence of any psychosis in girls who are continuously under pressure at school and in the parental home. In addition, an acquired aversion towards everything sexual may also be an etiological factor. Often this is combined with obesity or emaciation (THOMÄ, 1961). These women are psychologically and psychosexually infantile. The estrogen secretion is decreased in the presence of normal gonadotropin excretion. Differentiation from primary ovarian amenorrhea, which is considerably more common, is achieved by careful psychiatric exploration and perhaps in retrospect after successful psychiatric therapy. Often, however, the situation can only be clarified by an ovarian biopsy.

Primary amenorrhea can also be due to *dysfunction of one of the endocrine organs* correlated with the hypothalamo-hypophyseal-ovarian axis. Hypothyroidism and hyperthyroidism, as well as severe metabolic disturbance in unstabilized juvenile diabetes, can lead to primary amenorrhea, probably due to ovarian insufficiency caused via the hypothalamus.

States of *hypercorticism* produce primary amenorrhea more frequently than the endocrine and metabolic disorders mentioned. Thus the *adrenogenital syndrome* in the congenital or acquired forms can lead to primary or secondary amenorrhea if untreated. As a rule, however, it is the intersexuality and not the amenorrhea which leads to further investigation. The syndrome has been discussed in detail elsewhere (p. 358, PRADER). When virilization arises only after the 2nd or 3rd year of life, the presence of an adrenocortical tumor must be considered.

According to present-day knowledge the so-called pre- and postpubertal forms of the adrenogenital syndrome are also congenital disorders of steroid metabolism in the adrenal cortex. Borderline adrenal hyperplasia usually results in secondary amenorrhea (p. 593).

Cushing's syndrome arising before the menarche leads to primary amenorrhea if not treated. This symptom, however, retreats into the background beside the basic symptoms.

Endocrine active ovarian tumors (arrhenoblastoma, hilus-cell tumor, adrenal-rest tumor, chorioepithelioma and perhaps also tumors of the granulosa-theca cells) can cause primary amenorrhea. This is especially true of arrhenoblastomas. More often however, these tumors cause secondary amenorrhea (p. 638).

Primary amenorrhea is a concomitant symptom in the *Laurence-Moon-Biedl syndrome* (BIEDL, 1922, 1933; KEIFER, 1950), giving way to retinitis pigmentosa (which can lead to amaurosis), imbecility, dwarfism and obesity. Polydactylia, which is often present, and the involvement of siblings are evidence of the occurrence of this incurable neurological disease.

b) Secondary Amenorrhea

Secondary amenorrhea is more common than primary. Before endocrinopathies are considered, pregnancy and its sequelae must be excluded in an amenorrheic patient of sexual maturity, whatever the patient may say.

Physiological amenorrhea during the puerperium lasts a maximum of 3 months in a woman who is not breast feeding, and 4 months in the nursing mother.

Physiological amenorrhea also occurs frequently in the postmenarchal period, and is probably a reflection of a certain lability in the functional chain between hypothalamus, hypophysis and ovaries. Treatment is only exceptionally necessary since normal rhythm often arises spontaneously. Disturbed primary cycles, however, occur more frequently in women who later suffer from ovarian dysfunction. Secondary amenorrhea arising during the climacteric is due to the gradual development of generative and vegetative ovarian insufficiency. Menopause before the 40th year of life, is referred to as an *early menopause* (precocious climacteric). The climacteric syndrome is due to the reduced estrogen secretion (p. 582). At the same time there is a rise in gonadotropins. The cause of premature ovarian insufficiency is often not clear. Cytogenetic investigations have shown that an early menopause arises for example in patients with an XXX set of chromosomes (GORDON, 1967). In contrast to other women with precocious climacteric, these women often have hypogonadism.

The largest group of patients with pathologic secondary amenorrhea includes young women of inconspicuous appearance in whom psychological influences produce menstrual disorders. This is a form of hypothalamic amenorrhea, described as *psychogenic or emotional amenorrhea* on the basis of its etiology. Conflicting factors are at the root of the disturbance: unsatisfactory matrimonial or familial conditions, psychosexual tension, exaggerated fear of pregnancy, desire for a child, fears about the future, financial problems etc., and frightening experiences such as accidents, sudden death of a relative, war experiences. Conflicts arise from disturbed living conditions. Severe psychological trauma such as occurs during wars leads to amenorrhea particularly frequently. Examples of this are amenorrhea occurring during states of emergency, internment in concentration camps and ghettos, and during flight (WHITEACRE, 1944; SYDENHAM, 1946; STROINK, 1947; HEYNEMANN, 1948; TIETZE, 1948; BASS, 1947; MAYER, 1948; KNEER, 1949; NOCHIMOWSKI, 1946; NETTER, 1962). Secondary amenorrhea due to psychological factors was observed in up to half the female members of prison camps. Rapid development of secondary amenorrhea resulting from psychological factors belies the causal influence of malnutrition. Malnutrition begins to act only after several months but also contributes to the fact that 90% of the women in prisoner camps were amenorrheic. A purposeful reaction underlies this emotional amenorrhea: preservation of the species is abandoned in favor of self-preservation. This is also applicable to *nutritive amenorrhea* due to chronic inanition (KEYS, 1950; ZUBIRAN, 1953). In some women, quite trivial psychological influences are sufficient to produce menstrual disorders. The occurrence of secondary amenorrhea in girls and young women during a change in environment (time spent abroad in another part of the country), is a good illustration of this. On the other hand, not all

women react to even very severe psychological factors with the development of amenorrhea. Some do not react at all or react in some other way. We therefore speak of labile-cycle and stable-cycle women (SEITZ, 1939), which is meant to indicate that constitutional factors are involved. We know nothing about the nature of these individual differences. Women suffering from emotional amenorrhea often have immature and infantile personalities and unexpected experiences cause erroneous hypothalamic regulation. The hormone values vary widely and are dependent on the duration of amenorrhea. Urinary excretion of pituitary gonadotropins is decreased in most of these women, and may be completely absent in some. Estrogens are correspondingly reduced. Hypogonadotropism is rarely associated with hypothyroidism or hypocorticism. The HPG activity in the urine is quite commonly normal in the presence of normal or even elevated estrogen levels. High HPG and low estrogen levels can be found in acute forms, and the women affected complain of climacteric symptoms (RAKOFF, 1962). Excretion of metabolites of adrenal corticoids may vary widely in certain forms (STURGIS, 1962), and the urine excretion of 17-keto steroids may be considerably raised.

The observation that gonadotropin excretion is often reduced at least initially led to the conclusion that the hypothalamus is the main seat of the disorder in these cases of emotional amenorrhea. Any estrogen deficit in these women may be due to the fact that LH excretion is disturbed in the presence of normal or even raised excretion of FSH (KLINEFELTER, 1943). Reduced LH excretion has been described in psychogenic amenorrhea (MCARTHUR, 1958).

Amenorrhea with *anorexia nervosa* must also be classified as a hypothalamic form of amenorrhea (LASÈGUE, 1873; GULL, 1874; NEMIAH, 1950; BLISS, 1960; THOMÄ, 1960). This condition is not an endocrinopathy but primarily a psychoneurosis which takes the form of an aversion to any form of food intake. These patients are psychologically immature, and are incompatible to changes in environment. Domineering parents are often involved initially. The discontinuation of nutrition, which can lead to cachexia, results secondarily in limited gonadotropic function. Animal experiments have revealed that the release but not the production of gonadotropins is reduced during inanition. The reduced formation or absence of estrogens leads to atrophy of the reproductive organs and secondary sexual characteristics in these cases too.

Pseudocyesis must be classified in the group of psychogenic amenorrhea (MOULTON, 1942; FRIED, 1951; BENEDEK, 1952). It is an excellent example of the psychological influence on reproductive processes. It is a psychoneurosis arising from an exaggerated wish for a child or from pathologic fear of pregnancy. A series of indefinite signs of pregnancy arise: nausea, vomiting, meteorism, increase in weight with formation of striae distensae, enlargement of the breasts with lactation. Rhythmic uterine contractions start at the "calculated date" of delivery. In contrast to the other psychogenic forms of amenorrhea, secondary ovarian insufficiency does not arise in this case. Estrogen production is normal and atrophy of the genitalia fails to occur. Secretory transformation of the endometrium may even be well developed.

Because of the functional structure of the hypothalamus, it is not surprising that hypothalamic amenorrhea can be associated with disorders of other functions regulated by the central nervous system. Examples of these functions are: fat and carbohydrate metabolism, water balance and wake-sleep regulation. Thus, secondary amenorrhea is often found in obese women. This is probably not due to the obesity; both are probably concomitant consequences of the same psychological influence. Normalization of weight is followed quickly by the return of menstruation, so that one is tempted to assume a direct causal connection. However, it is the removal of the psychological disturbance which probably leads to the conscious limitation of food intake and often to the release of gonadotropins at the same time.

The patients are often unaware of the psychological factors causing the amenorrhea, and quite often emphatically deny them. Wide experience and great patience and tact are required for the doctor to expose the connection, which is often complex.

If neither psychological nor organic causes can be found to explain hypothalamic amenorrhea, it is justifiable to refer to an *idiopathic disorder*. These cases are probably examples of emotional amenorrhea where no psychogenesis can be found. Obesity is often present at the same time (BAHNER, 1969).

Analogous clinical illnesses have also been observed with tumors in the region of the tuberal part of the hypothalamus. On the basis of animal experiments it is suspected that this area is responsible for regulation of body weight. However, in the majority of cases, there are hypothalamo-hypophyseal functional disorders of unknown etiology. The high familial incidence suggests the influence of hereditary factors. The fat distribution is typically feminine, fat deposition being mainly in the buttocks, thighs, lower abdomen, breasts and shoulder

girdle. The distal extremities on the other hand remain quite slim. The psychological structure of these women is in keeping with the somatic composition in that they are calm, well-balanced people. Although the patients are of normal height, the distal regions of the extremities are quite short. This irregularity in bone growth indicates a central dysfunction arising early. External and internal genitalia are hypoplastic (secondary hypogonadism). Menarche is not usually delayed, but the primary cycles are often abnormal, in the form of menorrhagia with oligomenorrhea. Amenorrhea finally develops. Hypertrichosis is often visible on the lower jaw, extremities and lower abdomen but is not very marked. Pituitary gonadotropin levels are diminished in the urine, in contrast to the elevated values in primary hypogonadism, and normal levels of the total gonadotropins in polycystic disease of the ovaries. Estrogens are reduced. The endometrium can show a certain degree of proliferation so that the progestin test gives a positive result. The endometrium is usually atrophic after amenorrhea of long duration.

In addition to these psychoneurotic forms of amenorrhea, hypothalamic amenorrhea has also been observed in association with *true psychoses*, especially those of depressive character (BLEULER, 1954). Secondary hypothalamic amenorrhea can also be *iatrogenic* for example after administration of sexual steroids, and particularly depot-progestins. Amenorrhea has also been reported during the administration of reserpine, chlorpromazine, barbiturates, morphine and dithiocarbamide (Fig. 47, p. 572).

A special form of hypothalamic dysfunction leads to the *Chiari-Frommel syndrome* (CHIARI, 1852; FROMMEL, 1882; GUMPEL, 1960; JAZMAN, 1963; KAISER, 1963; HASKINS, 1964; CHARD, 1964). This syndrome consists of postpartum amenorrhea with galactorrhea, often associated with moderate obesity. Gonadotropin and estrogen levels are reduced. The syndrome is due to increased prolactin secretion by the anterior pituitary, possibly resulting from the loss of the inhibitory factor in the hypothalamus, and associated with a reduction in the release of gonadotropins at the same time (CANFIELD, 1965). (The reader is referred to p. 556 for a discussion of the prolactin-inhibiting factor.) The uterus and ovaries atrophy due to the decrease in FSH and LH secretions. Secondary sexual characteristics are not affected by the estrogen deficit. The increased prolactin excretion has been demonstrated biologically with pigeon goiters. With the exception of the ovaries, function of the other endocrine organs is not altered.

The case histories of these women often show an exaggerated state of fear in connection with delivery so that psychological factors could very well be involved in the etiology of this syndrome (MÜLLER, 1962). This has been confirmed by the observation of a Chiari-Frommel syndrome arising after a pseudocyesis. The Chiari-Frommel syndrome together with special cases of Forbes-Albright syndrome (Ahumada-del Castillo or Argonz-del-Castillo syndrome) may form a group of hypothalamic forms of amenorrhea due to common psychogenic etiological factors, where no organic changes can be found in the region of the hypothalamus and pituitary. However, even in the puerperal form, the possibility of an intracranial neoplastic process must always be kept in mind (see below).

The Chiari-Frommel syndrome is too frequently diagnosed. The two mandatory symptoms of amenorrhea and galactorrhea can be caused by a series of drugs such as the "pill", reserpine, phenothiazine, α-methyl dopa and Tofranil (HOOPER, 1961; RABINOWITZ, 1961; KLEIN, 1964), in cases where the frequence of the galactorrhea is directly dependent on the amount of drug administered. Both symptoms have been observed together in cases of hypothalamic and hypophyseal neoplasms, hypothyroidism [sometimes combined with precocious puberty (VAN WYK, 1960; BROWN, 1965)], and after hysterectomy (SACHS, 1959).

The Chiari-Frommel syndrome must be differentiated from the considerably more common *uncomplicated postpartal ovarian insufficiency*, which is due simply to generative ovarian insufficiency. Estrogen excretion is normal and progesterone administration leads to bleeding from the secretory transformed mucosa. There are no features of failure. 17-Ketosteroids and 17-OH corticoids are often at the upper normal limits, particularly in obese women. Adrenal and thyroid functions are normal. The etiology and pathogenesis of this disorder are still not clear. It is possible that there is a refractory connection between the ovaries and gonadotropic stimulation, such as is present in lactating women (KELLER, 1968). There are no investigations about the gonadotropic activity in series of these women.

Secondary amenorrhea is often the first symptom of a pituitary adenoma and craniopharyngioma in the adult woman (ROGERS, 1963). At the onset of the illness, there is only a loss of gonadotropic function, but later the clinical picture of panhypopituitarism develops. Loss of hypothalamic function (loss of formation and release of the gonadotropin-releasing factor, and other hypophysiotropins) can arise due

to pressure on the centers lying between the supra-optic and paraventricular nuclei when the tumor is situated more anteriorly, or through compression of the eminentia mediana and tuber cinereum when the tumor is more posteriorly placed. The suprasellar tumor lying anteriorly causes pressure on the optic chiasma resulting in visual disturbances (homonymous hemianopsia).

The *Forbes-Albright syndrome* (Ahumada-del Castillo or Argonz-del Castillo syndrome) (p. 106, Chap. V), in contrast to the Chiari-Frommel syndrome, arises independently of a birth. It is often caused by a chromophobe adenoma of the pituitary. The clinical features are secondary amenorrhea with galactorrhea, low values of urinary HPG and an estrogen deficit (AHUMADA, 1932; ARGONZ, 1953; FORBES, 1954; BRICAIRE, 1958; JAGIELLO, 1960; BERCOVICI, 1963).

The less common, endocrine-active eosinophil and basophil pituitary adenomas can also lead to secondary amenorrhea. This, however, retreats clinically into the background against the disorders of growth and metabolism (acromegaly, Cushing's disease).

"Compensation neurosis" must be considered as the cause of a secondary amenorrhea occurring after cerebral concussion. Postpartum amenorrhea may be due to Sheehan's syndrome (p. 92, Chap. V).

Women with *primary ovarian insufficiency* account for only a small proportion of patients with secondary amenorrhea. Patients with secondary ovarian hypoplasia, and a small number of women with an XXX set of chromosomes belong to this group. This form of amenorrhea has already been mentioned under primary amenorrhea.

Patients with *polycystic disease of the ovaries* must also be discussed in this section (p. 609 ff.). Since this disease is a pluriglandular disorder of uncertain etiology and pathogenesis, classification under ovarian amenorrhea is entirely arbitrary. The form described originally by STEIN and LEVENTHAL (1935) referred often to obese young women with hirsutism, secondary amenorrhea or oligomenorrhea, sterility, and enlarged polycystic ovaries.

Epidemic parotitis may occasionally lead to *ovarian destruction* causing primary or secondary amenorrhea. On the other hand, the functional capacity of the ovaries is very impressive, since menstrual disturbances may be completely absent even in cases where bilateral ovarian tumors have caused almost complete pressure atrophy of the ovarian stroma. The one exception to this is the Krukenberg tumor, which commonly gives rise to secondary amenorrhea when present bilaterally.

Endocrine-Active Ovarian Tumors. Arrhenoblastomas, tumors of the hilus cells, adrenal remnant tumors, chorioepitheliomas, and less frequently tumors of the granulosa-theca cells can give rise to amenorrhea. This is especially true of the arrhenoblastoma (p. 638).

This tumor arises most frequently between the 15th and 30th years of life. Defeminization and masculinization are the predominant clinical features, and are due to prolonged action of the slightly increased testosterone production. Excretion of the 17-ketosteroids is usually at the upper normal limit or slightly increased, and never reaches values as high as those found with adrenocortical tumors. The excretion values are generally also below those in the adrenogenital syndrome. Estrogens are usually normal, while gonadotropin excretion is decreased.

Dysfunction of Related Endocrine Organs such as the adrenal cortex and thyroid gland can cause secondary amenorrhea. Thus, the *postpubertal* form of the adrenogenital syndrome is a further cause. The probability of an adrenocortical tumor increases with advancing age of onset of this syndrome. Like hyperfunction, hypofunction in Addison's disease can also lead to secondary amenorrhea. The same is true of hypo- and hyperthyroidism.

The borderline cases of adrenal hyperplasia are of more practical importance since the incidence is greater. Mild cases can lead simply to a shortened luteal phase, whereas severe cases can give rise to anovulatory cycles with secondary amenorrhea. These cases of adrenal hyperplasia are a type of postpubertal adrenogenital syndrome where there is only a slight or moderate increase in the excretion of 17-ketosteroids and where the pregnanetriol excretion is also only slightly elevated. These women usually present clinically with hirsutism. Enlargement of the clitoris may also be present. There is a mere generative ovarian insufficiency in most of these cases. Progesterone leads to withdrawal bleeding.

Traumatic or atretic amenorrhea is a rare form of secondary amenorrhea (*Fritsch-Asherman syndrome, métrose de réceptivité Moricard*, so-called uterine adhesions or synechia) (ASHERMAN, 1948; 1950; 1957). It is a uterine amenorrhea in which gonadotropins and ovarian sexual steroids are normally secreted and formed, in which the cyclic sexual processes run a normal course but menstruation fails to occur. It is usually due to a too radical curettage post partum or post abortum, if it was performed especially in the first 14 days after termination of a pregnancy. Less frequently, manual removal of the placenta with mechanical re-

moval of the basal stratum of the endometrium or chemical or radiogenic cauterization for severe hemorrhage may result in traumatic amenorrhea. Tuberculous endometritis may exceptionally cause this type of amenorrhea by causing partial or total adhesion of the two uterine walls.

Secondary amenorrhea has also been observed after postoperative adhesion of the cervical canal, e.g. after amputation of the cervix during a prolapse operation, a diagnostic or therapeutic conisation, or electrocauterization of an ectropion.

Adhesions of the vagina after cauterization giving rise to amenorrhea are rare.

False amenorrhea is caused by all these forms of secondary gynatresia. The molimina menstrualis quickly leads the patient to the doctor.

c) Diagnostic Procedure (Tables 43, 44)

Amenorrhea is not a disease but merely a symptom. It must therefore be investigated thoroughly before treatment can be started. Often, however, treatment is only symptomatic. Unscrupulous symptomatic therapy is very often not only useless, but associated with the danger of indirect damage, as it may mask the disease underlying the amenorrhea. As long as there is no obstruction (gynatresia) in the discharging genital organs, primary amenorrhea should not be investigated or treated before completion of the 18th year of life. Spontaneous menarche has only exceptionally been observed after this age [approximately 0.3% of cases of primary amenorrhea (GRIMM, 1968)]. An investigation should then be instituted as promptly as possible, since the woman's future depends to a considerable extent on the prognosis.

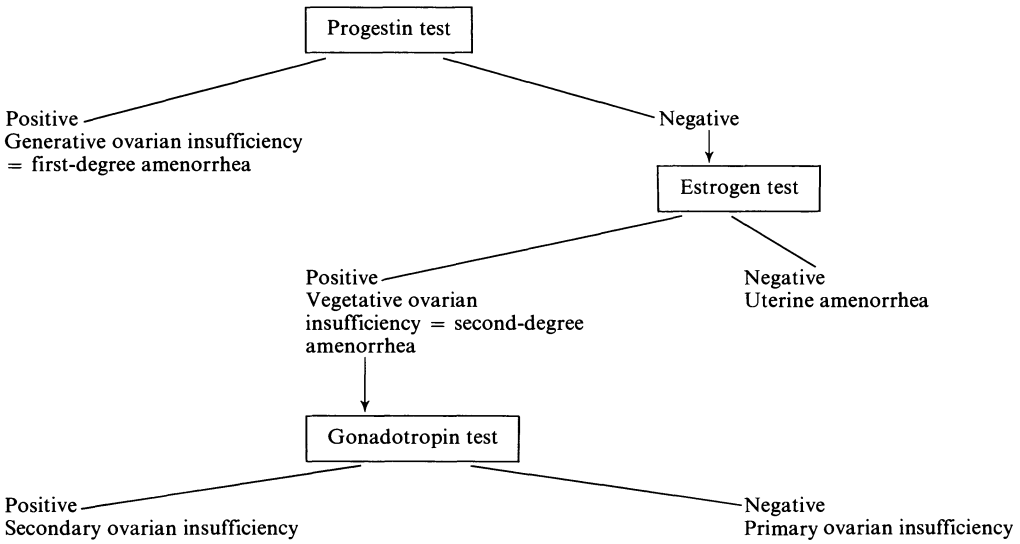
Physiological amenorrhea must be excluded before any form of treatment is started. Every amenorrheic sexually mature female must be considered pregnant until the opposite has been proved. A pregnancy can be diagnosed or be excluded with a high degree of certainty (96–98%) by means of the immunological pregnancy test as early as 14 days after the

Table 43. Diagnostic interpretation of amenorrhea

A. Case history
General and gynecological examination

B. Basal temperature, Shorr smears, cervical mucus,
HPG activity in 24-h urine

C. Hormone tests



D. Estrogens, pregnandiol, pregnantriol, 17-ketosteroids, 17-OH corticoids
T_4, iodine uptake, basic metabolism
Methyrapone, dexamethasone, and ACTH tests
Ocular fundus, perimetry
Idiogram
Endometrium biopsy, culdoscopy or laparotomy, ovarian biopsy
X-ray examinations: bone age, sella turcica, colpogram, hysterosalpingogram, urogram, etc.

Table 44. Diagnostic procedure in amenorrhea

Examination	Symptoms	Primary amenorrhea	Secondary amenorrhea
1. Case history	Familial incidence	Testicular feminization Laurence-Biedl-Moon syndrome (agonadism) Adrenogenital syndrome	Adrenogenital syndrome
	Postpartum occurrence (after collapse, mental trauma, curettage)		Sheehan's syndrome Chiari-Frommel syndrome Asherman's syndrome
	Previous infection (genital TB, parotitis epidemica)	MORICARD's syndrome (métrose de réceptivité)	
	Conflict situations shock	Emotional amenorrhea	
	Brain injury (cerebral concussion, contusion)	Postconcussion amenorrhea (compensation neurosis)	
	Slow-release progestagens, inhibitors of ovulation, reserpine, phenothiazines		Iatrogenic amenorrhea
2. General condition	Obesity, pronounced	Laurence-Biedl-Moon syndrome	Cushing's syndrome
	Obesity, moderate		Stein-Leventhal syndrome Chiari-Frommel syndrome
	Cachexia	Anorexia nervosa	
	Dwarfism	Adrenogenital syndrome Gonadal dysgenesis	
	Body hair as in man	Adrenogenital syndrome Virilizing ovarian tumor	
	Absence of pubic and axillary hair	Testicular feminization	
	Scanty growth of pubic hair, lateral portions of eyebrows scanty		Sheehan's syndrome
	Breasts Hypoplastic or atrophic	Primary and secondary ovarian insufficiency	
	Galactorrhea		Chiari-Frommel syndrome Ahumada-del-Castillo syndrome Iatrogenic: reserpine, phenothiazines (usually without amenorrhea) Hypothyreosis Acromegaly
	Inguinal hernia	Testicular feminization	
3. Genital status	No uterus present Vagina blind	Testicular feminization	
	No vagina present Uterus rudimentary	Mayer-Rokitansky-Küster syndrome	
	Vagina hypoplastic	Primary ovarian insufficiency	
	Clitoris hypertrophic	Adrenogenital syndrome Virilizing ovarian tumor Hermaphroditus verus Testicular feminization	
4. Hormonal status	HPG normal (LH lowered) Estrogens lowered Progesterone test negative Estrogen test positive	Hypothalamic amenorrhea (see Table 40) Exceptions: some forms of emotional and nutritional amenorrhea (Release blocked)	
	HPG lowered Estrogens lowered Progesterone test negative Estrogen test positive	Pituitary amenorrhea (see Table 40)	

Table 44 (continued)

Examination	Symptoms	Primary amenorrhea	Secondary amenorrhea
	HPG elevated Estrogens lowered Progesterone test negative Estrogen test positive	Ovarian amenorrhea (see Table 40)	
	HPG normal Estrogens normal Progesterone test negative Estrogen test negative	Uterine amenorrhea (see Table 40)	
	HPG normal Estrogens normal (occasionally lowered) Progesterone test positive	Polycystic ovary	
	HPG lowered Estrogens lowered Progesterone test positive	Adrenogenital syndrome	
	Urinary steroids lowered		Sheehan's syndrome Panhypopituitarism with pituitary tumors M. Addison
	17-ketosteroids elevated	Psychogenic amenorrhea Adrenogenital syndrome in adrenocortical hyperplasia Polycystic ovaries Virilizing ovarian tumors	
		Adrenogenital syndrome due to adrenocortical tumors	
	Estrogens lowered	Ovarian insufficiency (primary and secondary) Testicular feminization	
	Estrogens normal	Polycystic ovaries Adrenogenital syndrome Mayer-Rokitansky-Küster syndrome	
5. Vaginal cytology	Atrophic	Ovarian insufficiency (primary and secondary) Testicular feminization	
	Normal i.e. good estrogen effect	Polycystic ovary Traumatic amenorrhea (Asherman's syndrome) Métrose de réceptivité (Moricard's syndrome)	
6. Culdoscopy or Laparotomy	Uterus		
	None present	Testicular feminization	
	Rudimentary	Mayer-Rokitansky-Küster syndrome	
	Hypoplastic	Gonadal dysgenesis Ovarian hypoplasia	
	Ovaries		
	None present	Agonadism Testicular feminization	
	Hypoplastic or rudimentary		
	Threadlike	Gonadal dysgenesis (gonadal anlage, streak)	
	Cylindrical	Ovarian hypoplasia	
	Large, pale	Polycystic ovaries	
	Oystershell-like		
7. Radiographic examination	Urography		
	Malformations	Gonadal dysgenesis Mayer-Rokitansky-Küster syndrome	
	Colpohysterography		
	Synechiae	Gynetresia	Traumatic amenorrhea
	Sinus urogenitalis	Adrenogenital syndrome	
8. Chromosomal sex	Negative ("male")	Gonadal dysgenesis ♂ Testicular feminization Genuine hermaphroditism XX woman	

first missed period. Charting the basal body temperature is cheaper—a hyperthermal phase lasting for more than 16 days indicates a pregnancy with the same high probability as the immunological pregnancy test (BARTON, 1945).

Pathologic amenorrhea necessitates thorough investigation. A careful case history and the general and gynecological examinations usually provide valuable information on the etiology and pathogenesis (Table 44). Gynatresia and genital malformations, such as the Mayer-Rokitansky-Küster syndrome, can be detected or excluded (molimina menstrualis), and the presence of gonadal dysgenesia, testicular feminization or adrenogenital syndrome can further be indicated.

A biphasic basal body temperature in an amenorrheic patient indicates uterine amenorrhea, whereas the smear and cervical mucus show the degree of estrogen production.

The chromosome idiogram is often valuable in primary amenorrhea. Between 28–40% (JACOBS, 1961; PHILIP, 1965) of these patients have a chromosome anomaly. Estimation of gonadotropic activity (HPG) in the 24-hour urine permits differentiation between hypergonadotropic amenorrhea and hypo- or normogonadotropic amenorrhea. The hypergonadotropic form reflects primary ovarian insufficiency and allows substitution therapy at the most. Ovulation cannot be induced, and treatment of the sterility is thus hopeless.

The reactive capacity of the endometrium is examined by the *gestagen test* (Table 45), which should be performed before the *estrogen test* (Table 45). Both tests are negative in the presence of uterine amenorrhea. The result of the gestagen test permits generative ovarian insufficiency (Stage I or mild degree) to be differentiated from the severe form of vegetative ovarian insufficiency (Stage II or severe degree).

The use of progestagens in the gestagen test is not only simpler and cheaper, but also produces better defined withdrawal bleeding within 2–4 days after discontinuation due to the rapid fall in the hormone level. The development of withdrawal bleeding after withdrawal of the hormone (positive gestagen test) indicates estrogen formation in the ovaries and a certain degree of endometrial proliferation. Therefore only a generative ovarian insufficiency is present due to failure of ovulation. This disorder of the function of the hypophyseal-hypothalamic system may be caused by endogenous factors (i.e. through a dysfunction of a related endocrine organ).

If the gestagen test is negative, a subsequent estrogen test is a further means of investigation (Table 45).

Table 45. Testing the reactivity of endometrium or ovaries

A. Gestagen test
a) Oral Progestagen (Primolut-N; Gestanon, etc.) e.g. 3 × 2 5-mg tablets of Primolut-N daily for 5 days Withdrawal bleeding within 2–4 days
b) Parenteral Depot gestagen i.m. e.g. Proluton-Depot (250 mg) Lutocyclin M (200 mg) Withdrawal bleeding within 10 days Oily solution: 10 mg/day for 5 days i.m.
B. Estrogen test
a) Oral Ethinyl estradiol e.g. Progynon-C 3 × 1 0.02-mg tablet daily for 14 days Withdrawal bleeding after 2–4 days
b) Parenteral Depot estrogen i.m. e.g. Progynon-Depot 20 mg (2 ampoules) Ovocyclin M 20 mg (2 ampoules) Withdrawal bleeding after 4–5 weeks
C. Gonadotropin test 75 IU HMG/day for 10 days (HMG: Pergonal; Humegon) 5000 IU HCG/day on days 10, 11, and 12 (HCG: Pregnyl; Gonadex; Primogonyl) If negative, repeat with double these dosages

Uterine amenorrhea is present if withdrawal bleeding does not occur within 4–5 weeks after parenteral administration or after 8 days at the latest after discontinuation of the tablets (negative estrogen test). In such a case, the endometrium is not reacting. A positive estrogen test with a negative gestagen test shows the presence of an endometrium capable of reacting, associated with almost complete failure of the formation of ovarian estrogens (vegetative ovarian insufficiency, ovarian insufficiency grade II or severe degree). The question as to whether primary or secondary ovarian insufficiency is present can be answered by estimation of the urinary gonadotropins. Elevated levels (hypergonadotropic amenorrhea) are found in primary ovarian insufficiency. Nuclear sex, chromosome idiogram, laparotomy, and gonadal biopsy are further methods of investigation of the disorder. Secondary normogonadotropic or hypogonadotropic ovarian insufficiency requires further thorough investigation of the basic disease to detect any grave organic disorders in the hypophyseal-hypothalamic region (intracranial, particularly intrasellar tumors) or of other endocrine organs (Cushing's disease, Addison's disease, hypothyroidism, hyperthyroidism, diabetes mellitus). It is useful for neurologist, ophthalmologist, and endocrinologist to cooperate (Table 45).

The *gonadotropin test* (Table 45) is used to examine the reactive ability of the germinal parenchyma in the presence of secondary ovarian insufficiency. It is of prognostic significance only. If there is no ovarian reaction, there is no chance of normalizing the ovarian cycle. The test is expensive and necessitates daily checks of the size of the ovaries to avoid overstimulation. The amount of gonadotropin must be adjusted individually.

d) Prognosis and Therapy

The prognosis of amenorrhea is primarily dependent on the type of underlying disease. If the basic disorder can be removed cycles often arise spontaneously. This is not only applicable to organic disorders but also to psychogenic disturbances, which are much more common. In assessing the degree of success of treatment it must be remembered that a spontaneous cure not infrequently arises.

STAEMMLER (1964) found the following figures for normalization among his patients:
in hypothalamic dysfunction 49%, 25% of these spontaneously; in postpartum ovarian insufficiency 54%, about half spontaneously; with hypoplastic ovaries 23%, no spontaneous cures; with polycystic ovaries 46%, 8.5% of these spontaneously.

Whereas all other illnesses concerned in amenorrhea show approximately the same recovery rate, patients with hypoplastic ovaries have a definitely worse prognosis. The number of spontaneous cures is particularly high for postpartum amenorrhea, whereas a spontaneous recovery is not to be expected with hypoplastic ovaries, and the chances of such a cure are very slight with polycystic ovaries. The prognosis of the amenorrhea is dependent on the basic disorder and the duration of the amenorrhea. Thus, lasting success is estimated in a mean of 55% of cases of amenorrhea of 1 year's duration and less, in 49% in cases of 1–3 years' duration, and 29% where amenorrhea has lasted for more than 3 years (STAEMMLER, 1964). The prognosis improves with the extent of estrogen proliferation in the endometrium before the onset of treatment.

Often, the causal factor cannot be found or cannot be removed. Treatment in such cases is aimed at the production of an ovarian or an endometrial cycle, according to whether or not there is any desire for children. *Gynatresia* is treated surgically. The operation should be performed as early as possible, before fertility is permanently damaged. In the presence of hymenal atresia the hematocolpos only should be evacuated, and patients should be treated with antibiotics to reduce the high risk of infection. The uterus empties spontaneously.

Careful dilatation of the uterine cavity or of the cervix, together with parenteral estrogen and progesterone treatment, are sometimes sufficient in cases of *uterine synechia* or *cervical stenosis*. The curative effect of the hysterosalpingography (as has been described for traumatic amenorrhea) is due to the same mechanical action. Homotransplantation of mucosa through the intracavital autotransplantation of one tube (STRASSMANN, 1935) is reserved for special individual cases.

Treatment of *congenital vaginal aplasia* consists in surgical construction of a vagina allowing intercourse. Numerous methods are available. We find that the surgical construction of an artificial vaginal lumen lined with free epidermal flaps has proved satisfactory (modified method after KIRSCHNER, WAGNER and MCINDOE) (BÄBLER, 1967). The operation should only be done if regular sexual intercourse can occur subsequently. This factor among others also determines the results of the operation.

Patience and sympathetic listening alone can lead to success in cases of *psychogenic amenorrhea*. Consultations with the husband, parents or superior at work may be necessary. The cyclic employment of sexual steroids to produce withdrawal bleeding frequently cannot be avoided (Table 49b). General measures such as relief from work, sufficient sleep, physical exercise and reduction in weight can restore menstruation in some cases, but are of a supportive nature in all cases. Severe psychoneurosis such as anorexia nervosa requires treatment by a psychiatrist and hospitalization for treatment of the electrolyte disturbance often present.

Three principle forms of hormone treatment are available:

stimulation at the hypothalamic or ovarian level,

substitution at the uterine and vaginal levels, and

central inhibition with subsequent "rebound effect".

An ovarian cycle is achieved through ovarian stimulation with either gonadotropins or clomifene.

There may be a slight chance of producing a so-called rebound effect (increased release of pituitary gonadotropins) by hypophyseal-hypothalamic suppression by means of sexual steroids and their discontinuation.

An endometrial cycle is obtained by substitution treatment.

α) Stimulation Therapy

Ovarian stimulation can be achieved either indirectly with clomifene or directly with gonadotropins. This form of treatment is only indicated when ovulation is to be induced in cases where a child is wanted. Stimulation is thus the form of treatment for anovulatory sterility.

The quantitative HPG excretion is of prime importance in the prognosis and choice of treatment. The excretion pattern can be used for assessment of the LH peak. It is now possible to determine the excretion pattern without particular exertion by immunological estimation of LH. On the other hand, quantitative excretions can still only be determined by considerably more costly biological and radioimmunological tests. Treatment is adjusted primarily to the excretion type.

Table 46. Stimulant treatment with clomifene citrate. (See also p. 630ff.)

Special precaution:
Only to be used in the presence of a functional hypothalamo-hypophyseal system and ovarian tissue.

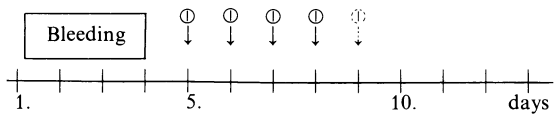
Endocrinological:
Normogonadotropic oligo-amenorrhea or anovulation; estrogens normal.

Standard therapy:
50–100 mg Clomid/day for 4–5 days.

Preparation:
Dyneric, Clomid (MERRELL)

Ovulation (in about 70% of patients):
On average 8–12 days after beginning of therapy.

50–100 mg/day



Risks (lower than with gonadotropins):
Overstimulation with formation of ovarian cysts with torsion of the pedicle, rupture and intra-abdominal hemorrhage, Meigs's syndrome, thromboembolism.
Multiple ovulation: multiple births (7.5%)

Implications:
Bimanual gynecological examination every other day during stimulation.
Discontinuation of the treatment if there is rapid growth of the ovaries.

Less dangerous than stimulation with gonadotropins, but also less effective.

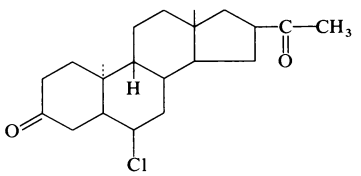
Treatment is less costly and time-consuming. No lasting success.

Four main gonadotropin excretion types have been demonstrated with anovulation:

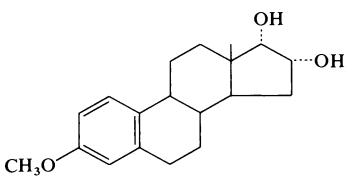
a) If the *anovulation* is *normogonadotropic* a trial of diencephalic stimulation with Dyneric (clomifene citrate) or Sexovid F 6066) is justified (Table 46). In order to reduce the risk of over-stimulation as much as possible, which however is lower than with gonadotropins, the initial dose of clomifene should not exceed 50 mg daily. If the ovarian stimulation obtained with this dose is inadequate, a daily dose of 100 mg for 4 to 5 days may induce ovulation. The danger of over-stimulation is especially great when treatment lasts for longer than 10 days or is repeated. A new cycle of treatment should only be started at the earliest after 4 weeks and after pregnancy and ovarian cysts have been excluded (GREENBLATT, 1966; SCHREINER, 1968).

The LH peak achieved with Dyneric may exceptionally be too low to produce ovulation. In such cases, additional administration of HCG can lead to success (Fig. 60). Determination of the excretion pattern is valuable in such cases. Recently steroids like Retroid (Roche), a weak progestagen, and Epimestrol (Organon), a weak estrogen, have been discussed for the induction of ovulation.

The former is 1,6-dehydro-6-chloro-retroprogesterone, the latter 3-methoxy-17-epiestriol (Fig. 59). Both steroids seem to act as a positive feedback by enhancing the LH secretion (KELLER, 1971).



Retroid



Epimestrol

Fig. 59. Symptoms of the menopause

We found a fairly good effect when 4–8 mg Retroid was given daily for 10 days beginning on day 15 of the cycle, or 5 mg Epimestrol daily for 10 days from the 5th day of the cycle onward (Table 46a).

In cases of secondary amenorrhea ovulation was found in 29% treated with Retroid and 26% treated with Epimestrol (KELLER, 1971).

Table 46a. Stimulant therapy with steroids. (See also p. 630)

Special precaution:
Only to be used in the presence of a functional hypothalamo-hypophyseal system and ovarian tissue.

Endocrinological:
Young women with normogonadotropic oligo-amenorrhea or anovulation: estrogens normal.

Standard therapy:
Retroid: 4–8 mg/day for 10 days, beginning on day 14–16 of bleeding.
Epimestrol: 5 mg/day for 10 days, beginning on day 5 of bleeding.

Preparations:
Retroid (Roche), Epimestrol (Organon).

Not dangerous, less effective than clomifene citrat.

Table 47. Stimulant therapy with human gonadotropins. (See also p. 629)

Special precaution:
Only to be used in the presence of functional ovarian tissue.

Endocrinological:
Hypo- or normogonadotropic oligo-amenorrhea or anovulation, estrogens normal or lowered.

Basis of treatment:
HMG + HCG

Preparations:
HMG: Pergonal (Istituto Serono, Rome)
Humegon (Organon, Oss)
HCG: Pregnyl (Organon)
Gonadex (Leo)
Primogonyl (Schering)

Ovulation:
On average 12–60 hours after beginning of HCG medication.

Risks:
Overstimulation of the ovaries: formation of cysts with torsion of pedicle, rupture and intra-abdominal hemorrhage, ascites, pseudo-Meigs's syndrome, thromboembolism.
Multiple ovulation: multiple births.

Implications:
1. Daily bimanual gynecological examination. Discontinuation of treatment if ovaries grow rapidly;
2. daily hormonal vaginal cytology (karyopyknotic index)
3. daily assessment of cervix factor:
Appearance and quantity of cervical mucus
Spinnbarkeit
Fern-leaf test
Possibly Sims-Huhner test;
4. Basal temperature

Advantage:
Most effective form of stimulant therapy

Disadvantages:
Dangerous, costly, time-consuming for patient and doctor; no lasting success.

b) The treatment of choice for induction of ovulation in cases of *hypogonadotropic anovulation* is direct stimulation with HMG/HCG. The ideal patient for treatment with human gonadotropins must fulfill the following conditions: sterility, age less than 35 years, normal nonfunctioning ovaries, no endogenous gonadotropin production and normally developed genitalia (GEMZELL, 1965). Normal or slightly reduced gonadotropin secretion in the presence of anovulation does not exclude treatment with human gonadotropins, but these cases are more difficult to treat since the reactive capacity of the ovaries varies widely. They generally react more sensitively to HMG. This is especially true of polycystic ovaries, and wedge resection or treatment with clomifene is therefore an adequate form of therapy in such cases. Pergonal: 75 IU FSH + 75 IU LH (Instituto Serano, Rome) and Humegon: 75 IU FSH (Organon, Oss) are HMG preparations. The simultaneous action of FSH and LH provided by Pergonal and Humegon is essential for follicular growth and the production and secretion of estrogens. Almost pure forms of FSH or LH have only a slight action on the follicle.

The "LH-spurt" required for releasing ovulation is achieved by giving HCG in addition (Pregnyl, Primogonyl, Gonadex).

We have found the *2-phase treatment* the most satisfactory *schedule for administration* (Fig. 60). The woman receives 75–150 IU HMG daily for 10–12 days, followed by HCG. Thus a total of 1000 IU FSH is administered on average. This roughly corresponds to the amount of FSH secreted by the anterior pituitary before ovulation (p. 562). HCG is given in three equal daily doses of 5000 IU (Fig. 60). Treatment with HCG can follow or overlap that with HMG. If the ovaries are enlarged and painful after the last injection of HMG, we refrain from giving HCG. HMG treatment is continued until a significant estrogen effect can be observed in the vagina and in the cervix in particular. This stage is usually reached after 8 to 10 days, and is also indicated by the estrogen excretion which must be estimated constantly (GEMZELL, 1969).

If the follicles do not respond to this form of stimulation the doses can be gradually increased.

Very often 150–225 IU HMG daily are needed for ovarian stimulation.

Ovulation usually occurs between 12 and 60 hours after the initiation of HCG treatment.

The form of administration advocated by CROOKE (1967) has not proved so satisfactory in our opinion (KELLER, 1969). This form of treatment consists of an initial single dose

of HMG, varying between 5 and 15 ampoules of Humegon or Pergonal. At the point when the estrogen effect is at its maximum, generally after 8 to 10 days, 5000 IU of HCG are given daily for 1–4 days (Fig. 60). BETTENDORF (1962) was able to induce ovulation with HPG alone.

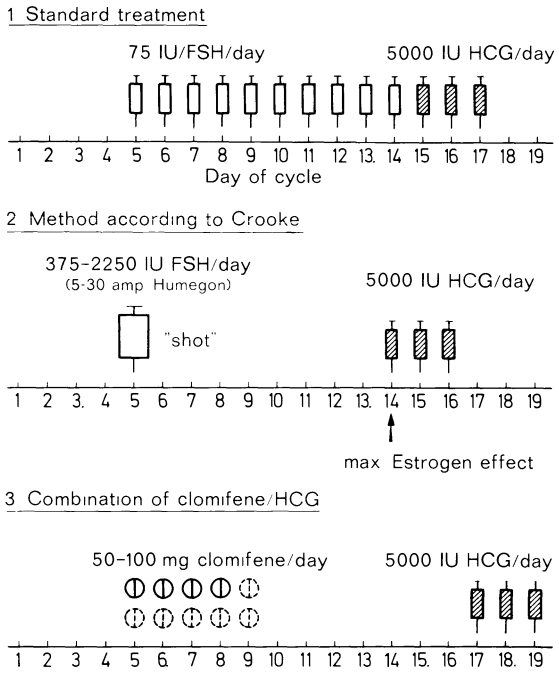


Fig. 60. Administration schedule for human gonadotropins

According to the results it is supposed that the effect of HMG on the growth of the follicle follows the "all-or-nothing" law. The range between the dose which is without effect and the one which provokes an overstimulation is very small. According to the excretion of estrogens there is always an overstimulation of the ovaries by the exogenous HMG (GEMZELL, 1969).

The results connected with the induction of a biphasic ovarian cycle are very good in correctly selected patients. Ovulation can almost always be induced by this method, and pregnancy occurs in 40–45% of these previously sterile women (VAN DE WIELE, 1965; GEMZELL, 1969). About a third of these pregnancies, however, terminate in abortion or in premature birth, mainly because of multiple pregnancies. About 25% of the children born alive are from multiple pregnancies (Table 48).

Patients must be subjected to a daily bimanual vaginal examination during treatment with human gonadotropins to avoid the development of large polycystic ovaries. GEMZELL (1969) has recommended daily estimation of urinary estrogens and pregnanediol and of progesterone in the blood, but this is expensive and only justified in the investigation of special scientific problems. It is unnecessary during treatment and the karyopycnotic index and cervix factor are sufficient for estimation of the estrogen effect (Table 48).

Table 48. Results obtained in 228 women with primary or secondary amenorrhea with human hypophyseal FSH and HCG over a period of 8 years (1960–1967). (After GEMZELL, 1969)

1960–1967	
No. of patients	228
No. of treatments	463
Pregnancies	101
Including: twins in	19
triplets in	5
Abortions or premature births	30

c) In cases of *hypergonadotropic anovulation* the prognosis is considerably less favorable (repeated finding of more than 2 mg in 24 hours). The ovaries in such cases are already under increased gonadotropic stimulation. As long as the values are not too high (over 3 mg IRP/24 h), an attempt can be made to induce ovulation by means of diencephalic stimulation with clomifene. Additional gonadotropic stimulation is not indicated.

d) When hormone levels are quantitatively normal, reduced follicular response to gonadotropic stimulation can be suspected. This state may be present postpartum (KELLER, 1968). Here too, an attempt to increase central ovarian stimulation with clomifene is permissible.

β) Substitution Therapy (Table 49)

Substitution therapy is indicated when induction of withdrawal bleeding by an effect at the endometrial level is desired or when an estrogenic and gestagenic effect is to be produced on the primary and secondary sexual characteristics.

Spontaneous cycles can be induced in exceptional cases where the hypothalamo-hypophyseal-ovarian axis is intact, by stimulation of the hypothalamo-hypophyseal system caused by rhythmic administration and withdrawal of estrogens and gestagens. Treatment must, however, be continued for at least 6 months. Estrogens and progesterone can be given orally or parenterally in injections. Oral administration of gestagens has the advantage that the fall in hormones occurs rapidly, thus allowing the onset and end of the withdrawal bleeding to be well defined and treatment to be short.

One effect of the administration of estrogens and gestagens is the suppression of gonadotropin secretion. Experiments with animals show that there is an excessive release of gonadotropins from the pituitary after the discontinuation of long-term administration of sexual steroids. This is described as the *rebound effect* (HOHLWEG, 1934). It causes increased ovarian stimulation. Although it has never been proved that gonadotropin release in the female is increased by this treatment, there have been frequent attempts to release ovulatory cycles by hormonal inhibition of the hypophysis (BUSCHBECK, 1957). Simultaneous administration of high doses of estrogens and gestagens over a period of weeks produces the most pronounced suppressant effects on hypothalamus and hypophysis (Table 49).

Table 49. Substitution therapy

A. If genital bleeding is desired
Only possible in the presence of a responsive endometrium
a) If some estrogen stimulation is still present and the amenorrhea is of only short duration (< 1 year):
Oral gestagen e.g. Primolut-N (120 mg)
3 × 2 tablets (30 mg) daily on days 21–24 of cycle, or Primosiston
3 × 1 tablet on days 17–26 of cycle,
or Duogynon
1 tablet daily for 2 days (24 and 25 of cycle).
b) Modified Kaufmann schedule:
3 × 1 tablet of Progynon C daily for 14 days, followed by
3 × 1 tablet of Primosiston daily, later from day 5 of cycle
3 × 1 tablet of Progynon C daily for 10 days, followed by
3 × 1 tablet of Primosiston daily for 10 days, or Sequens, Estirona for 20 days from day 5 of cycle.
c) To induce pseudocyesis
10 mg of a slow-release estradiol preparation on each of days 1, 10, 20, and 30 (Ovocyclin M; Progynon-Depot)
Oral Progestagen (e.g. Primolut-N)
days 11–20 1 tablet daily
days 21–30 2 tablets daily
days 31–40 3 tablets daily
B. If uterine bleeding is unnecessary, estrogens alone can be given:
0.25 mg stilbestrol/day
0.02 mg ethinyl estradiol/day
2.50 mg Premarin, Presomen, Progynova 1–2 mg/day
10 mg slow-release estrogen (1–2/month) (Ovocyclin M; Progynon-Depot)

Substitution therapy with estrogens in gonadal dysgenesia (rudimentary gonads) is aimed at preventing osteoporosis, stimulating skeletal growth and developing primary and secondary sexual characteristics. Complete growth compensation is, however, not achieved (OVERZIER, 1969). Whether cyclic genital bleeding is desired will depend on the woman's mentality. It is important that the patient is given to understand in the early stages that the sterility is permanent and not wait until she gets married. Normal ability to take part in sexual intercourse and a normal libido can be attained. Substitution should not be started before the 12th year (WILKINS, 1957). It corresponds to the long-term treatment presented in Table 49 under Form Ab or B.

There is no evidence, as has been claimed (STANGE, 1958), that dysgenetic gonads are more prone to tumors (HAUSER, 1961). Removal of such gondas is therefore unnecessary. Psychological instruction and advice are the important factors in these patients, since subsequent mental maturation often occurs with substitution treatment (POLLOCK, 1955).

A few authors recommend bilateral orchidectomy in cases of *testicular feminization* because of the alleged increased danger of malignant degeneration in retained testes (STANGE, 1958). Substitution therapy is absolutely essential in these castrated patients since severe symptoms of failure may arise. We were not convinced of the necessity of castration in our cases (SCHREINER, 1959); we leave the testes intact or remove one testis if a herniotomy is necessary.

Treatment of *ovarian hypoplasia* consists of stimulation with human gonadotropins where there is a desire for children (Table 47). Where children are not wanted, treatment consists of substitution (Table 49). Substitution with estrogens is adequate as long as uterine bleeding is not necessary for psychological reasons (Table 49).

Uterine hypoplasia can be favorably influenced by stimulation producing a pseudocyesis (Table 49).

The treatment of polycystic ovaries is discussed on p. 614ff. If a tumor can be excluded, prolactin release can be suppressed in the *Chiari-Frommel or Forbes-Albright syndrome* with high doses of estrogens or progestagens, over 3–4 months (HASKINS, 1964). Cyclic substitution with sexual steroids in normal doses leads to uterine bleeding but has no significant influence on the galactorrhea (MCDONALD, 1965). There have been reports of success with normal doses of clomifene (p. 599) giving rise to ovulatory cycles, and even of pregnancy (KAISER, 1963; LORAINE, 1966).

There is no causal treatment possible for Sheehan's syndrome, except if pregnancy occurs. Pregnancy causes physiological hypertrophy of the residual anterior pituitary, and since this hypertrophy does not usually regress postpartum, improvement is obtained. One must however be aware that this method of treatment is restricted and dangerous in these patients. A new sub- or postpartum complication to

which these patients are particularly liable can cause complete destruction of the residual anterior pituitary and thus lead to an extremely severe form of global insufficiency of the anterior pituitary lobe. Long-term treatment is usually purely symptomatic. Estrogens in doses which do not induce genital bleeding are adequate to prevent genital atrophy and osteoporosis (Table 49B). The production of cyclic bleeding is sometimes necessary for psychological reasons in young women (Table 49b). Substitution with human gonadotropins is only exceptionally considered as a form of treatment (p. 584). Knowledge of this can, however, be of psychological importance to younger patients, since this method provides at least a theoretical possibility of pregnancy.

2. Dysfunctional Uterine Bleeding

Dysfunctional uterine bleeding indicates abnormal functional endometrial bleeding resulting from primary or secondary ovarian dysfunction (synonyms: functional uterine bleeding, metropathia hemorrhagica).

The bleeding can be of various types. Cyclic bleeding disorders can be so-called temporal anomalies (= impaired bleeding rhythm) or type anomalies (= disorder of type of bleeding), or the bleeding may be acyclic (metrorrhagia) or a combination of different anomalies. Anovulatory bleeding is common.

The causal factor leading to bleeding disturbances is endocrine in nature and can lie anywhere on the cortex-limbic system-hypothalamus-hypophysis-ovary-endometrium axis or less commonly in the adrenal cortex or thyroid gland. Endocrinopathies are exceptionally the cause. Disturbed ovarian function however is always the core and presents mainly as anovulation. Investigations in experimental animals and observations in castrated women have yielded information on the pathogenesis of functional bleeding disturbances. Castration of a sexually mature female monkey leads to endometrial bleeding within 2–5 days, regardless of the point in the cycle at which castration was performed (E. ALLEN, *in* OBER, 1957). Thus estrogen withdrawal alone is sufficient to induce bleeding; but the endometrium is more sensitive to and reacts more quickly to withdrawal of progesterone, even after short-term, slight progesterone effects. Clinical observations in castrated women have confirmed these results (OBER, 1957). Furthermore, it has been deduced that increasingly high doses of estrogens are necessary to maintain endometrium proliferation and prevent breakthrough bleeding over a longer period of time. The picture of glandular cystic hyperplasia arises in the endometrium following the continuous action of estrogens alone for at least 4 weeks (p. 607). The quantitative estrogen influence is also essential in the determination of the histological picture and of the type of dysfunctional bleeding. Glandular cystic hyperplasia occurs only if the total estrogen excretion is more than 30 µg/24 h for at least 3–4 weeks, even in the presence of fluctuating estrogen production (Fig. 61). Amounts between 10–30 µg/24 h results in simple proliferation (Fig. 61). If estrogen administration is not increased after a certain time, a state of relative estrogen deficiency arises, resulting in constant bleeding termed as breakthrough bleeding (W. M. ALLEN, 1951), which occurs from the surface where the blood is coagulable.

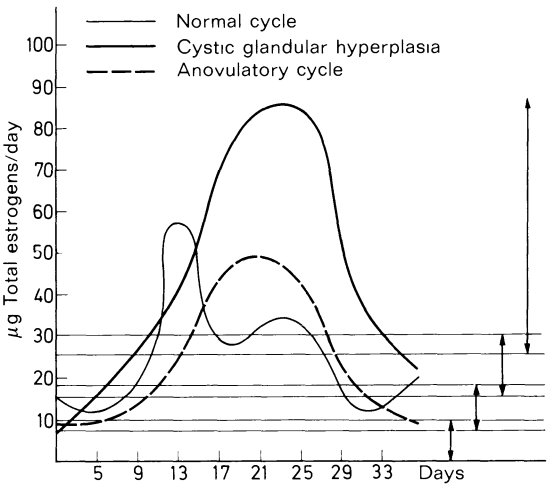


Fig. 61. Quantitative changes with fluctuating influence of estrogens on the endometrium. (After BROWN, 1959)

Increasing amounts of progesterone are also required to maintain the secretory transformed mucosa. This fact is illustrated by the hormone treatment of endometriosis (p. 633). Atrophy and shrinkage occur only under the influence of progestin, whereas relative progestin and estrogen deficiency does not give rise to a typical mucosal destruction but to superficial bleeding.

Further observations have shown that withdrawal of progesterone even after only one day results in withdrawal bleeding without histologically detectable endometrial changes. Destruction of the functionalis occurs characteristically only when progesterone acts for longer than 10 days together with estrogens. This is probably associated with the facts that destruction of menstrual mucosa necessitates the development of a characteristic vascular

system, consisting particularly of spiral arteries, and that the process of development requires at least 10 days.

All the investigations mentioned have shown that functional or dysfunctional endometrial bleeding occurs if the estrogens and progestins required for build-up are withdrawn or are present in inadequate amounts. This fact is of practical importance in the treatment of dysfunctional uterine bleeding.

Dysfunctional bleeding accounts for a considerable proportion of the female patients subjected to curettage for bleeding disorders. Dysfunctional bleeding is responsible for 60% of all diagnostic curettages performed in the Gynecological Department in Zurich. The disorder can arise at any age between menarche and early postmenopause, but is more frequent during the postmenarche and the premenopausal period. This is understandable, since luteal ovarian function is in a state of development or decline during these phases of life (DÖRING, 1963). Anovulatory cycles are also more frequent at these times (Table 58, DÖRING, p. 623). Of our patients with dysfunctional bleeding, 46% were over 40 years old. Other authors have found an incidence of about 66% (ISRAEL, 1967). In contrast, among our cases, only 1% of dysfunctional bleeding occurred in women under 20 years. This figure is consistent with those of other investigators (SUTHERLAND, 1953; ISRAEL, 1967). Incomplete abortion is the predominant reason for the bleeding in the majority of these young patients. Dysfunctional bleeding was present in only every fifth patient in this age group (SOUTHAM, 1960). These figures for young women are, however, misleading, since dysfunctional bleeding in this age group now only exceptionally requires curettage. Dysfunctional bleeding in young women, termed as "juvenile bleeding", can be very profuse, and if untreated can lead to severe anemia within a short time. The fertility of these patients is reduced even when the cycle later becomes normal (FRIES, 1947; SOUTHAM, 1966). However only 55% of these women later have a normal cycle, and normalization occurs in about half of them within 4 years (SOUTHAM, 1966). The risk of developing a carcinoma of the body of the uterus later seems to be greater in young women who suffer from dysfunctional bleeding (SOUTHAM, 1966). Dysfunctional bleeding is uncommon in women between 20 and 40 years, and the reasons are usually emotional. Bleeding due to disturbed gestation or neoplastic changes is predominant in this age group. Carcinoma of the uterus and dysfunctional bleeding top the list of conditions to be considered in the differential diagnosis of bleeding disturbances in the age group between 40 and 50. During the postmenarche and postmenopause, ovaries with numerous cysts 1–3 cm in diameter are the most frequent cause of dysfunctional uterine bleeding. The functional state of the ovaries is dependent on the state of the theca cells found in the cysts.

The histological picture of the endometrium is not only dependent on the age of the patient but also on the time at which the tissue is removed, i.e. the previous duration of bleeding. Mucosa in the phase after bleeding has occurred no longer indicates the original functional structure. Since anovulation is the principle reason for dysfunctional bleeding, the endometrium usually shows an exclusively estrogenic effect. All transition phases are observed, from weak proliferation through simple hyperplasia to glandular cystic hyperplasia and polyposis. A certain progesterone influence cannot be excluded in individual cases, since some glycogen and mucus are sometimes found in the endometrium. It is possible that the theca cells in the cystic cells become luteinized. When bleeding occurs from a fully secretory transformed endometrium, the possibility of an early abortion must be considered since about 40% of fertilized ova are unviable (HERTIG, 1948). Frequently no evidence of this can be obtained in the first two weeks of pregnancy, since the very small ovum can escape histological identification and the pregnancy test may still be negative. In a few cases, the endometrium shows secretory transformed areas together with regions purely proliferative in character (so-called irregular maturation, after TRAUT and KUDER, 1935), or the endometrium may show the picture of delayed regression of the corpus luteum (so-called irregular shedding). Atrophy of the endometrium is seen in only about 5% of cases (ZONDEK, 1954) (Table 50).

Before a diagnosis of dysfunctional bleeding can be made, neoplastic and inflammatory uterine changes and pregnancy must be excluded. The pretherapeutic investigation involves a fractional curettage first of all, which has a therapeutic effect in about 50% of cases. Curettage is essential in cases of bleeding in the premenopause. "Hormonal curettage" is of prime therapeutic and diagnostic importance particularly in younger patients (p. 607). If this procedure is unsuccessful dysfunctional bleeding is unlikely. Important aids to diagnosis are the basal temperature and pregnanediol excretion, which together with the pregnancy test help to clarify the question of a pregnancy. The case history often leads to a diagnosis, but differention from abortion may be difficult at times. A widely dilated os, clear cervical

Table 50. Histology of the endometrium in dysfunctional bleeding

Diagnosis	KISTNER (1964) (400 patients)	SUTHERLAND (1950) (861 patients)	Universitäts-Frauenklinik, Zurich (1968) (704 patients)
Hyperplasia	123	265	93
Atrophy	7	10	206
Irregular shedding	9	13	–
Devascularized mucus membrane	31	26	42
Simple proliferation	230	547	363

mucus that can be drawn into threads, and a positive fern-leaf phenomenon exclude a pregnancy. Disturbances of blood coagulation are of practically no importance in patients not receiving anti-coagulant treatment.

a) Disorders of the Bleeding Rhythm (Temporal Anomalies) (Figs. 62a and b)

These disorders present as oligomenorrhea or polymenorrhea. Since about 95% of all cycles last between 21–35 days between the ages of 20 and 40 (AREY, 1939), the term oligomenorrhea is applied when the cycle lasts for more than 35 days but less than 90 days (see amenorrhea, p. 585). Polymenorrhea occurs when the cycle last less than 21 days.

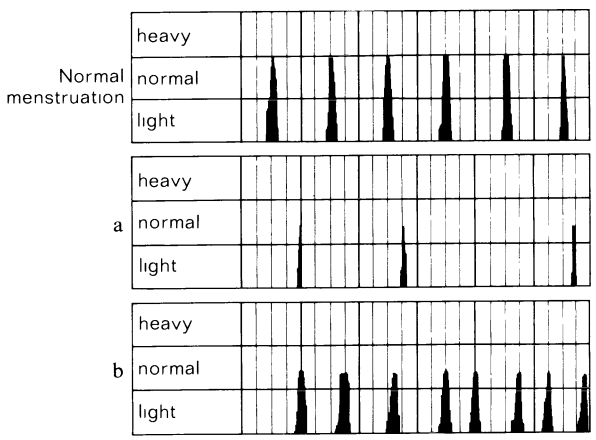


Fig. 62a and b. Dysfunctional bleeding. Anomalous rhythm (temporal anomalies). a) Oligomenorrhea = abnormally infrequent menstruation (interval >35 days, <90 days). b) Polymenorrhea = abnormally frequent menstruation (interval <21 days)

Most temporal anomalies result from hypothalamic dysregulation.

Oligomenorrhea is usually due to anovulation and less commonly to a biphasic cycle with a prolonged phase of follicular maturation. It can precede secondary amenorrhea. This is indicated in particular when the interval lasts longer than 45 days and hypomenorrhea arises at the same time. Menopause is often premature in women with oligomenorrhea, due to ovarian insufficiency.

Bleeding may occasionally be due to hypothalamic dysfunction such as occurs with severe psychological stress, malnutrition or obesity, dysfunctional states of related endocrine organs and in the syndrome of polycystic ovaries.

Oligomenorrhea is not important as long as there is no desire for children. Treatment is only necessary in unwanted functional sterility. The therapeutic procedure corresponds to that for secondary amenorrhea (p. 590). In cases of obesity, reduction in weight is often sufficient to normalize the cycle. Absence of ovulation and a shortened follicular maturation or luteal phase can also lead to polymenorrhea (KNAUS, 1950; MOSZKOWSKI, 1962). Experience has shown that functional sterility is present when the luteal phase is less than 10 days. The blastocyste, which is implanted around the 7th postovulatory day, produces too little HCG at this stage to prevent regression of the corpus luteum. Since ovulation occurs on the 8th day of the cycle at the earliest (HARTMAN, 1936; BREWER, 1947; HERTIG, 1956) the cycle of 18 days is the shortest fertile cycle.

Polymenorrhea arises commonly in the postmenarche and the premenopausal period, since luteal function of the ovaries is often absent or deficient in these phases. A shortened cycle may arise between the ages of 20 and 40 as a result of severe physical or psychological stress. As long as it does not produce anemia, treatment is not necessary during the postmenarche and the premenopausal period.

In the absence of ovulation, there are cycles with relatively regular follicular breakthrough bleeding of short duration (SCHRÖDER, 1928). It must be mentioned that 4–8% of all women who menstruate quite regularly have anovular cycles (EFFKEMANN, 1939; ROCK, 1939; SIEGLER, 1951).

Treatment is indicated when polymenorrhea arises secondarily during sexual maturity, and if there is sterility in the presence of a desire for children. Treatment is adjusted according to the basal body temperature and the endometrial

histology. The temperature on waking also permits the detection of pseudopolymenorrhea, which may exceptionally be due to extensive ovulation bleeding. A healthy way of life with sufficient sleep and relaxation are often enough to normalize the shortened cycle.

If the object of treatment is to prolong the interval without bleeding and possibly to reduce the severity of the bleeding, a mixture of estrogens and progestagens (ovulation inhibitors) can be given from days 5–25 of the cycle. If sterility associated with anovulatory cycles is the dominating feature, treatment with clomifene or human gonadotropin is indicated (p. 629).

The "displacing effect" of estrogens on ovulation due to inhibition of gonadotropin secretion can also be applied to cases with biphasic cycles with a reduced follicular phase: 10 mg estradiol benzoate or valerianate is given intramuscularly on day 5 of the cycle, or 0.05 mg ethinyl estradiol is given twice daily or 0.5 mg diethyl stilbestrol daily from day 3–10 of the cycle.

Finally, if the luteal phase is too short, the second phase of the cycle can be prolonged by substitution treatment, by giving for example Primosiston (ethinyl nortestosterone acetate 2 mg + 0.01 mg ethinyl estradiol) three times daily from day 21 to day 26 of the cycle.

Stimulation with chorionic gonadotropin is only justified in the treatment of sterility. HCG is given three times in a dose of 5000 IU between days 20–24 of the cycle.

b) Disorders of the Type of Bleeding (Type Anomalies) (Fig. 62c)

In contrast to the temporal anomalies, which are usually of a dysfunctional nature, type anomalies, with the exception of hypomenorrhea, mostly originate from organic causes.

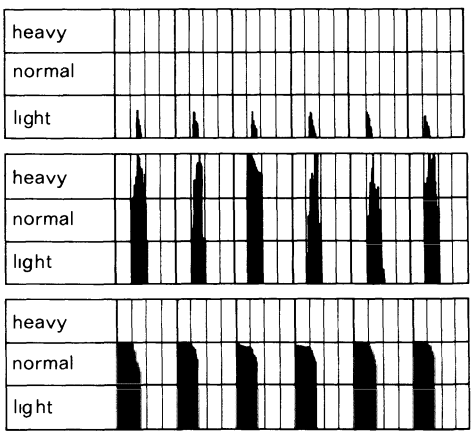


Fig. 62c. Anomalies in type of bleeding; hypomenorrhea = abnormally light menstrual bleeding; hypermenorrhea = abnormally heavy bleeding of normal duration; menorrhagia = abnormally prolonged menstrual bleeding (>7 days, <14 days)

In *hypomenorrhea* the bleeding is abnormally mild, and can present in the form of bleeding of short duration often lasting only a few hours or 1–2 days (LAX, 1939). Hypomenorrhea has been observed more frequently since ovulation inhibitors have been in use, and may precede amenorrhea. Like oligomenorrhea, with which it can be combined, hypomenorrhea may be due to hypothalamic primary ovarian dysfunction or to disorders of the correlated organs. It has also been observed in association with physiological stress, changes in surroundings and climate, obesity, the climacteric and in hypothyroidism and hyperthyroidism, Addison's disease, Cushing's syndrome and adrenogenital syndrome.

Treatment of hypomenorrhea is superfluous.

Hypermenorrhea is abnormally severe cyclic, uterine bleeding of 7 days' duration at the most. It is frequently characterized by the discharge of blood clots, since severe bleeding results in a relative deficiency of fibrinolytic enzymes in the decomposing endometrium. Hypochromic anemia develops with repeated episodes of hypermenorrhea. It is caused by organic changes in over 90% of cases. These changes cause a reduction in the contractility of the myometrium and an increase in the bleeding surface. Uterine hypoplasia, intramural or submucosal fibroids, polyps of the uterine mucosa, adenomyosis of the uterus and, exceptionally, bleeding diseases with clotting disorders may be involved. Psychological stress may occasionally give rise to hypermenorrhea.

Histology shows that the gestagenic influence on the endometrium is often inadequate and is removed during the bleeding.

Except during adolescence, a fractional curettage is indicated for diagnostic reasons. It is often of therapeutic value at the same time.

Hormonal treatment of dysfunctional hypermenorrhea is aimed at the improvement of secretory transformation, by giving, for example, 2 tablets Primolut-N 3 times daily between days 21 and 24 of the cycle, or at suppression of ovulation by giving an ovulostatic drug from day 5 to day 25 of the cycle.

Hemostasis of profuse dysfunctional bleeding is best achieved by giving Primosiston tablets in the following dosage: 5 tablets on the 1st day, 2 times 2 tablets on the 2nd day, 3 tablets daily from the 3rd to the 9th day (KAUFMANN). Withdrawal bleeding normally occurs 8 days after discontinuation of the treatment.

Menorrhagia indicates cyclic uterine bleeding lasting for more than 7 days but for less

than 14 days. The bleeding is often more severe than normal but can also be of normal severity; it is rarely diminished. If bleeding lasts for 14 days or more, then the term *meno-metrorrhagia* is used, since acyclic bleeding can be maintained in prolonged cyclic bleeding. Menorrhagia, and meno-metrorrhagia in particular, very frequently have organic causes. They can also arise as dysfunctional bleeding in combination with oligomenorrhea.

Endometrial hyperplasia occurs in the presence of a *persistent follicle* due to the prolonged action of estrogens (SCHRÖDER, 1928; 1954; BEHRENS, 1956). Follicular persistence may last a few days or several weeks but rarely longer than 8 weeks. A picture of *glandular cystic hyperplasia* develops in the endometrium when estrogenic influence lasts uninterrupted for more than 4 weeks (R. MEYER, 1913; SCHRÖDER, 1928). The histological picture is termed the "Swiss-cheese pattern" of the endometrium. Sooner or later, nutritional disorders arise in the endometrium due to the relative estrogen deficiency, and this in turn gives rise to metrorrhagia in the form of "spotting" or to true breakthrough bleeding in the form of metrorrhagia or meno-metrorrhagia. The amounts of estrogens produced by the ovaries are seldom above the bleeding threshold for longer than 8 weeks. Bleeding occurs from the surface, and the endometrial necrotic foci become progressively drained of blood over weeks in some cases. Severity and duration of the bleeding depend on the rate at which the estrogen level falls. Mucosa obtained from a curettage after several days of prolonged bleeding shows a "bled-out", poorly regenerating basalis.

Bleeding after follicular persistence is characteristic of the climacteric, due to the loss of generative ovarian function, and is termed as "juvenile bleeding" if between the 12th and 20th years. It arises during the postmenarche (FRIES, 1947; DEWHURST, 1963). The bleeding is often profuse in both cases and may lead to severe anemia. The prognosis for later normalization of the cycle and fertility is very poor (p. 604). The risk of the later development of carcinoma of the body of the uterus is elevated (SOUTHAM, 1966).

Oligomenorrhea and amenorrhea in the presence of follicular persistence are characteristic of patients with polycystic ovaries. Ovarian tumors producing estrogens (granulosa- and theca-cell tumor, occasionally Brenner tumors) can also lead to glandular cystic hyperplasia and breakthrough bleeding. The development of breakthrough bleeding in the late postmenopause should always suggest the presence of such a tumor.

The connection between glandular cystic hyperplasia and carcinoma of the body of the uterus is still not clear (TAYLOR, 1923; NOVAK, 1936; LARSON, 1954), and opinions are still divided in spite of numerous investigations (KISTNER, 1964). However, there is no doubt that there is a series of developmental stages, from glandular cystic hyperplasia to atypical adenomatous hyperplasia and carcinoma *in situ* of the endometrium. Thus, carcinoma of the body of the uterus has been observed to arise within 5 years in 12% of patients with atypical adenomatous hyperplasia (GUSBERG, 1958). Hypertension, obesity, diabetes and nulliparity (WEBER, 1961) are predisposing factors (so-called syndrome of carcinoma of the body of the uterus).

Delayed regression of the endometrium (so-called irregular shedding) produces dysfunctional bleeding. It is a special form of menorrhagia (SCHRÖDER, 1928; MCKELVEY, 1947; MCCLENNAN, 1952). This disorder is probably due to delayed regression of the corpus luteum. This is supported by persistent pregnanediol excretion, which maintains postovulatory values during the bleeding, and by the histology of the endometrium removed at the earliest 5 days after the onset of bleeding. The histology is characteristic: the endometrium shows signs of incomplete regeneration as well as secretory changes due to the prolonged progestative influence. This is in contrast with the situation in the normal cycle, where the regenerative phase has come to an end and the endometrium is beginning to show signs of proliferation by this stage.

In 9% of cases of meno-metrorrhagia arising between the ages of 10 and 20, and 20–30% of those occurring during the premenopause, there are organic changes (SUTHERLAND, 1953). The diagnosis should be confirmed by means of a fractional curettage before any therapeutic action is taken. The curettage may also have a therapeutic effect, since every type of dysfunctional bleeding is stopped at least temporarily. Juvenile bleeding in virgins is an exception. In these cases an attempt is made to stop the bleeding with hormone treatment. The same is applicable to recurrent dysfunctional bleeding occurring within 6 months after a curettage. Treatment consists of the administration of an orally effective estrogen-progestagen compound (e.g. Primosiston) in the following dosage (KAUFMANN):

1st day	5 tablets
2nd day	2 × 2 tablets
3rd to 9th day	3 × 1 tablet (total: 0.3 mg ethinyl estradiol + 60 mg ethinyl nortestosterone acetate).

The dysfunctional bleeding is stopped within 36 to 48 hours. Withdrawal bleeding from the secretory converted endometrium occurs 2–3 days after discontinuation of the tablets ("hormonal curettage"). Treatment with injections of an estrogen-progestagen compound (e.g. Primosiston) gives considerably worse results. The slow fall of the hormone level often leads to prolonged withdrawal bleeding.

About 50% of cases of juvenile bleeding and 60–75% of cases of glandular cystic hyperplasia recur during the premenopause (SOUTHAM, 1966; ISRAEL, 1967). Prophylaxis of recurrences necessitates plotting of the basal body temperature. If there is no rise in temperature within three weeks, there is a danger that the endometrium has become hyperplastic again. In this case, secretory transformation of the endometrium must be precipitated by the administration of exogenous progestin, because of the risk of recurrences. Oral progestagens are best used to produce transformation, for example in doses of 1 tablet of Primolut-N 3 times daily for 4 days (Table 21). Withdrawal bleeding occurs 2–3 days after discontinuation of the tablets. If bleeding does not occur, it can be assumed that the patient has reached the postmenopause (so-called progestin test, p. 597). Abdominal total hysterectomy and bilateral ovariectomy are indicated when glandular cystic hyperplasia arises during the postmenopause, since a minute hormone-active ovarian tumor may be present.

c) Acyclic Dysfunctional Uterine Bleeding (Fig. 62d)

Bleeding arising outside the cycle is termed as *metrorrhagia* or *additional bleeding*. It necessitates investigation by means of a curettage without exception since such bleeding is particularly likely to be due to organic changes. It can arise pre- or postmenstrually, and often leads to the clinical picture of menorrhagia. Dysfunctional premenstrual bleeding occurs in the presence of insufficiency of the corpus luteum due to a premature fall of the estrogen and progesterone levels. Insufficient secretory transformation of the endometrium, which is often associated with this condition, leads to functional sterility.

As has been mentioned already, the retarded regression of the corpus luteum and the delayed withdrawal of progesterone cause a delay in the menstrual rejection of the secretory transformed endometrium (irregular shedding), which may also present clinically as postmenstrual bleeding. The metrorrhagia is not really related to menstruation. It has recently become more

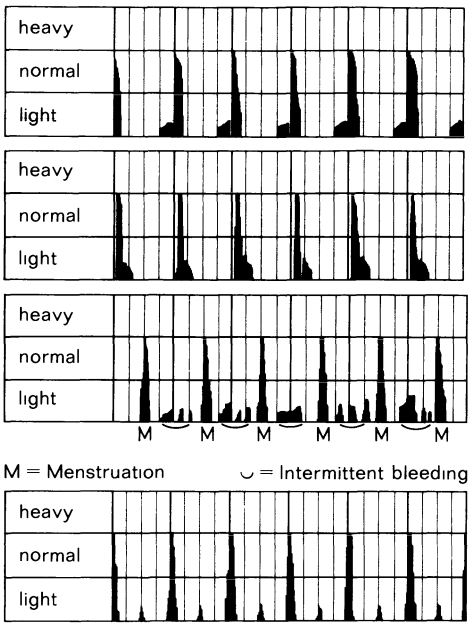


Fig. 62d. Acyclic uterine bleeding (metrorrhagia) = bleeding at times other than during the normal menstrual period: premenstrual bleeding, postmenstrual bleeding, intermenstrual bleeding = metrorrhagia, mid-cycle bleeding = ovulation bleeding = bleeding at the time of ovulation

frequent due to the use of ovulostatics (breakthrough bleeding).

Ovulation bleeding or *mid-cycle bleeding* is a special form of metrorrhagia. These terms should only be applied after organic causes have been excluded and the bleeding has been localized in the cycle by means of the basal body temperature and the possible development of mittelschmerz (DIDDLE, 1948). Ovulation bleeding lasting between a few hours and 2 days occurs in 20% of women with mittelschmerz. The benzidine test on cervical mucus is positive in as many as 94% of ovulating women at the time of ovulation (BROMBERG, 1956), so that very mild mid-cycle bleeding can be considered normal. If the bleeding is as heavy as during menstruation, it may simulate polymenorrhea (so-called pseudopolymenorrhea). It may exceptionally continue until the next menstrual period. It is believed to be a form of withdrawal bleeding due to the fall in estrogen secretion after ovulation.

Administration of 0.02 mg ethinyl estradiol daily from day 10–16 of the cycle in a 28-day cycle will compensate the postovulatory fall of estrogens, and thus prevent ovulation bleeding. If the bleeding still occurs, it is not ovulation bleeding. Premenstrual bleeding due to insufficiency of the corpus luteum is prevented by giving exogenous progestins: e.g. 1 tablet Primolut-N is given three times daily from

the 21st to 24th day of the cycle in a 28-day cycle. The rapid fall of progestin after intake of the tablets results in accelerated destruction of the secretory transformed endometrium, so that bleeding can be prevented even in cases of delayed regression of the corpus luteum. Frequently, only thorough curettage leads to arrest of the bleeding. Ovulostatics are used prophylactically against recurrence.

3. Polycystic Ovary

[Sclerocystic ovary, androgenic ovary (JEFFCOATE, 1964; SHORT, 1965), polycystic ovarian disease (GREENBLATT, 1960; GOLDZIEHER, 1963), Stein-Leventhal syndrome (STEIN & LEVENTHAL, 1935)].

In 1935 STEIN and LEVENTHAL described an apparently well-defined clinical syndrome consisting of sterility, amenorrhea and hirsutism in a group of young women, some of whom were obese, and all of whom had greatly enlarged cystic ovaries. Since then, numerous authors have reported almost identical cases, but more often similar clinical features with ovarian dysfunction. This makes the so-called Stein-Leventhal syndrome one of the most controversial syndromes in endocrinology, as far as its clinical features and pathophysiology are concerned. This syndrome now includes a spectrum of overlapping dysfunctions of the ovary and adrenal cortex, with clinical symptoms differing not only from one woman to the next, but also varying temporarily in the same woman. A few investigators have come to doubt the existence of this syndrome because of this extreme variability (NETTER, 1958; ROBERTS, 1960). The incidence of the syndrome differs greatly with different authors, depending on the criteria used to differentiate it from similar clinical syndromes. According to early reports it is rather rare. STEIN (1964) operated on only 108 cases within 34 years, and LEVENTHAL (1958) observed only 114 women with the so-called Stein-Leventhal syndrome in 29 years. Other authors report a considerably higher incidence (GOLDZIEHER, 1962; TAYMOR, 1963; PRUNTY, 1967).

Polycystic ovaries are found quite frequently, namely in 1.4% of a random sample of 12160 gynecological laparotomies (VARA, 1951), and in 3.5% of a consecutive series of 740 female autopsies (SOMMERS, 1956). Polycystic ovaries are encountered more frequently in sterile patients, as is to be expected (4.6%) (MCGOOGAN, 1954).

Thanks to improved methods of investigation which allow better understanding of the pathophysiology of the ovary it has been shown in the last ten years that quite a small fraction of patients suffer from the Stein-Leventhal syndrome, and that these patients can be definitely differentiated from a significantly larger group of women with polycystic changes in the ovaries (GOLDZIEHER, 1967).

Table 51. Symptomatology in 1079 published and confirmed cases with polycystic ovaries (GOLDZIEHER, 1962)

Symptom	Prevalence (%)	
	Mean	Range
Obesity	41	16–49
Hirsuties	69	17–83
Virilization	21	0–28
Amenorrhea	51	15–77
Sterility	74	35–94
Dysfunctional bleeding	29	6–65
Dysmenorrhea	23	–
Biphasic basal temperature	15	12–40
Corpus luteum found at laparotomy	22	0–71

Clinical symptoms vary. Consideration of 1079 surgically confirmed cases (Table 51) (GOLDZIEHER, 1962) suggests that *anovulation* is the most frequent symptom, arising on average in 85% of cases, but varying between 20% and 90% in the different series. Even ignoring the fact that the frequency of the diagnosis "anovulation" is partly dependent on the criteria used, the existence of this syndrome, which is characterized by a symptom which is often absent, is justifiably questionable. *Sterility* is found on average in about 75% of these women, and a previous pregnancy does not exclude the syndrome. Thus, for example, a woman can have two children with a short interval between after several years of anovulatory sterility and then become sterile again with oligomenorrhea or amenorrhea. *Secondary amenorrhea* is encountered in an average of 55% of patients, whereas primary amenorrhea is found only exceptionally. *Hirsutism* is quite common (70%) and is an important leading diagnostic symptom (GREENBLATT, 1968). *Virilization*, in contrast, is considerably less common, but occurs in 20% of patients. *Dysfunctional bleeding* can be very severe, and occurs in 29% of these women on average.

The typical patient is a young woman with hirsutism, in whom menarche arose at the right time (STEIN, 1964), but with a subsequent history of irregular bleeding at intervals of three to six months. The patient often seeks advice because of primary sterility.

The clinical findings connected with the development of the breasts, vaginal smears, cervical mucus, endometrial histology, and size of the uterus, are uncharacteristic, and can embrace the whole spectrum of clinical types of hypo- and hyperestrogenism.

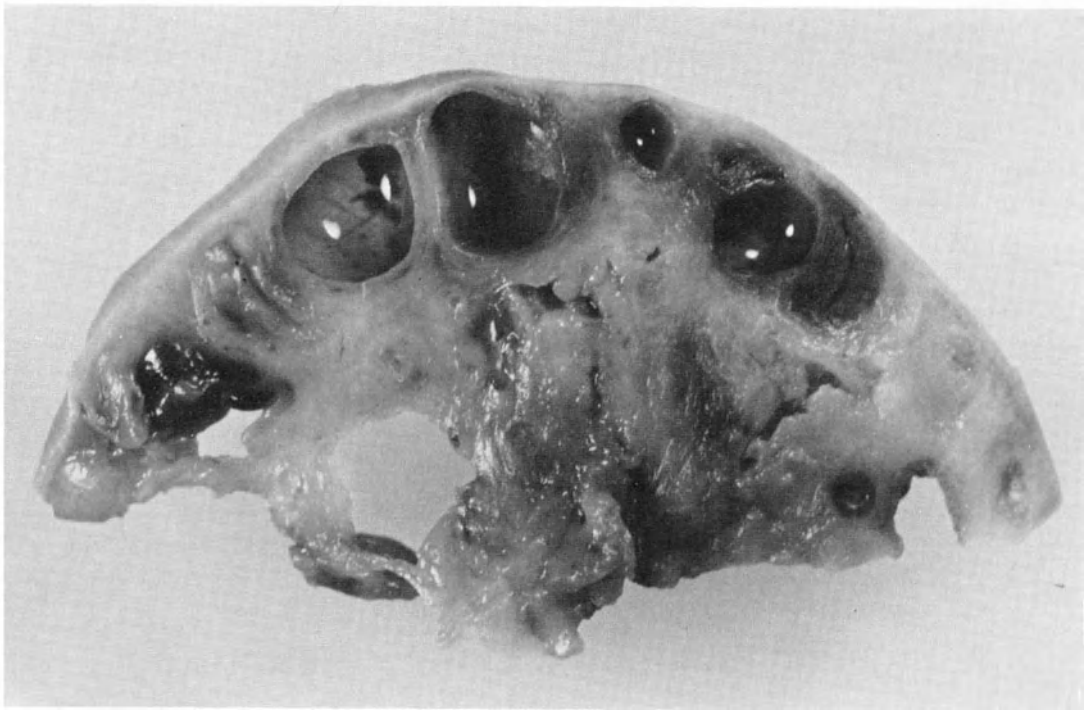


Fig. 63. Ovary taken from a patient with Stein-Leventhal syndrome. (After KRAUS, 1967)

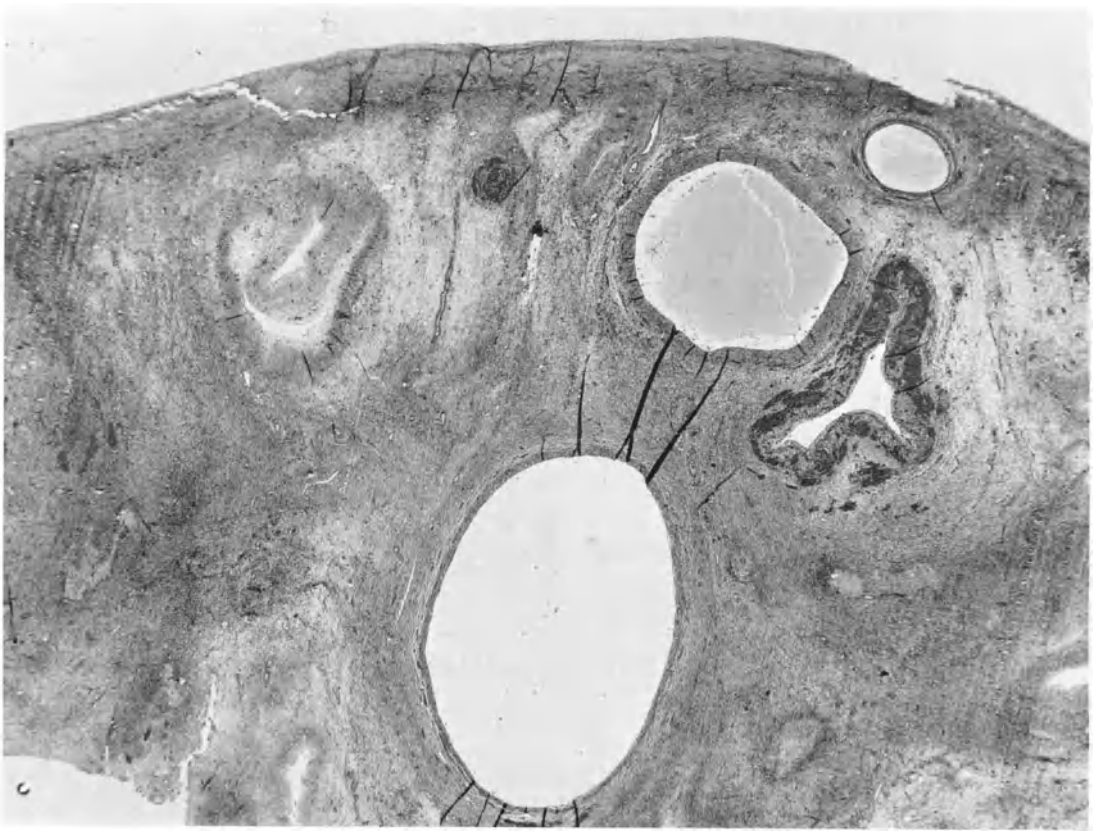


Fig. 64. Microscopic picture of ovary shown in Fig. 63. (After KRAUS, 1967)

The ovaries described originally by STEIN and LEVENTHAL (1935) were enlarged, with a maximum diameter of 4–6 cm. In some cases the ovaries were larger than the uterus. The same clinical picture is observed, however, in women with ovaries of normal size or with one enlarged ovary (EVANS, 1960; BENEDICT, 1962). Enlarged ovaries are found at operation in only 70–80% of the patients (GOLDZIEHER, 1962; TAYMOR, 1963; SMITH, 1965). The capsule is thickened and pearly-white in typical advanced cases (oyster shell-like ovaries), and the ovary is more vascularized than normal. The histological picture pathognomonic of the fully developed syndrome is characterized by fibrosis of the collagenous thickened capsule, which is a result of the influence of the androgens; there are numerous subcapsular follicular cysts and an increased number of atretic follicles; the follicular system shows a thickened and markedly vascularized theca interna with luteinized cells and frequent mitoses (PLATE, 1958; JONES, 1962; LEVENTHAL, 1963). It is generally assumed that the cells of the theca interna are the site where androgens are formed in normal or increased amounts (RICE, 1964). The histological picture thus indicates anovulation of long duration in the presence of strong and continuous gonadotropic stimulation. Like the clinical picture, the microscopical picture of polycystic ovaries varies considerably. The numerous subcapsular follicular cysts with diameters between a few millimeters and over one centimeter and the reduced number of atretic follicles are the only pathognomonic features. Fibrosis of the capsule has been described as characteristic by some authors (SHIPPEL, 1955; GOLDZIEHER, 1962; TAYMOR, 1963), but this has not been confirmed by other investigators (PLATE, 1958; JONES, 1962). Similarly, other authors claim that there is only an apparent increase in the cells of the theca interna, due to the increased number of atretic follicles (GOLDZIEHER, 1962; GREEN, 1965; ROBERTS, 1960; CHAMBERLAIN, 1964).

Corpora lutea or albicantia have been found in a mean of 22% of polycystic ovaries. This observation proves that ovulation occurs in a considerable number of cases of Stein-Leventhal syndrome (GOLDZIEHER, 1963).

There is a normal number of primordial follicles. The ovarian stroma is pale grey and edematous.

Hyperthecosis (thecomatosis) ovarii rarely occurs in association with the clinical picture of anovulation and hirsutism. In such a case there are large nests of thecal cells laden with lipids in the ovarian stroma (SHIPPEL, 1955; SCULLY, 1963). There are usually only a small number of cystic cortical follicles. The hyperplastic medullary stroma forms a tumor-like mass in the center. These ovaries are often associated with virilization, obesity, hypertension and symptoms of impaired glucose tolerance, all of which indicates adrenocortical hyperfunction. Analogous ovarian changes are observed during pregnancy.

Different forms of androgenic hyperplasia and tumors of the adrenal cortex can give rise to ovarian changes similar to those found in polycystic disease of the ovaries, corresponding to long-lasting anovulation in the presence of increased androgenic activity. There is, however, no enlargement of the ovaries; there are only a few cysts and the number of primordial follicles is reduced.

Increased formation of androgenic active steroids dominates the clinical picture in the syndrome of polycystic ovaries. In addition to the quantitative nature, the origin and biosynthesis of these androgens are of special interest.

A series of different investigations indicate that at least some of the increased amounts of androstenedione and testosterone are produced in the ovaries themselves. Incubation of tissue sections of polycystic ovaries gives rise to a significantly higher formation of androstenedione than in normal ovaries (LANTHIER, 1960; GOLDZIEHER, 1960; WARREN, 1962; ZANDER, 1962). It has also been demonstrated that the androsterone concentration in venous blood of polycystic ovaries is much higher than in normal circumstances in normal ovaries (MIGEON, 1960). Increased amounts of androstenedione and dehydroepiandrosterone were found in the follicular fluid of polycystic ovaries in the absence of 17-β estradiol (SHORT, 1961).

Observations of increased excretion of Δ^5-3 compounds such as Δ^5-pregnanediol and Δ^5-pregnanetriol (SHEARMAN, 1961; STERN, 1963) are of interest in connection with disturbed steroid synthesis of the ovaries. They indicate reduced activity of 3β-OH-dehydrogenase in polycystic ovaries.

On the basis of these investigations it is presumed that steroid synthesis deviates from normal in two ways in the polycystic ovary (Fig. 65):

a) decreased activity of 3β-OH-dehydrogenase and

b) reduced activity of the enzyme causing aromatization.

It is assumed on the basis of the ratios of H3 to C14 during the use of pregnenolone-7-H3 and progesterone 4-C14 in incubation experiments with polycystic ovaries (RYAN, 1965) that in the polycystic ovary steroids are

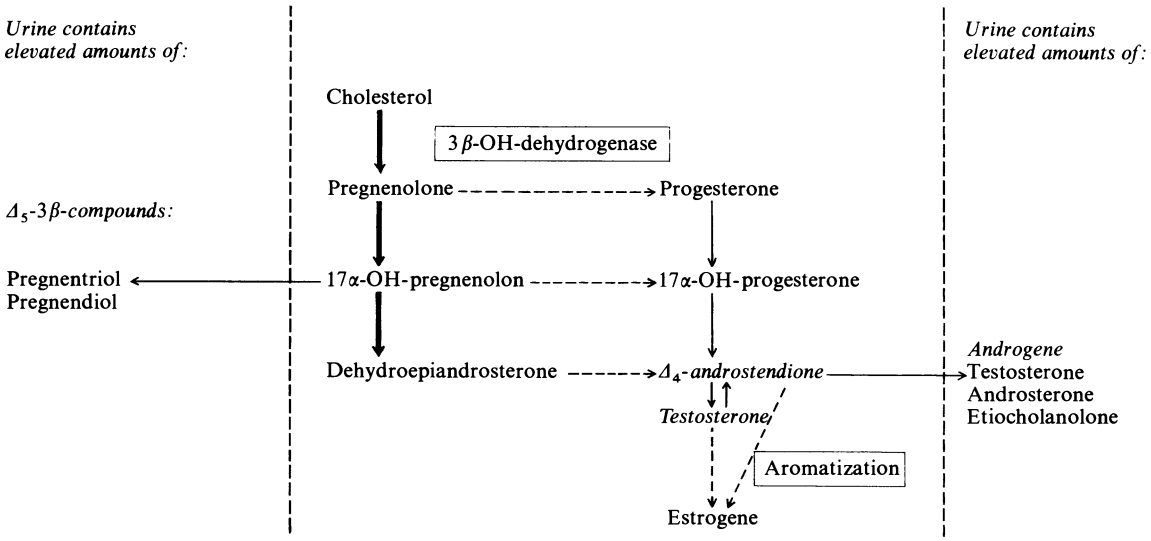


Fig. 65. Excretion of 17-ketosteroids and 17-hydroxycorticoids with the urine in women with polycystic ovaries

mainly synthesized via pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone and androstenedione (Fig. 65) (MAHESH, 1962). Thus, conditions are similar to those in the follicle of the normal ovary, the only difference being that there is an accumulation of androstenedione and testosterone due to the reduced activity of the enzyme causing aromatization of the A ring (SAVARD, 1965) (Fig. 65).

Similar conditions are also found in the ovarian stroma, and it is to be expected that both substances arise from the cells of the theca interna.

The involvement of the adrenal cortex in the syndrome of polycystic ovaries is still controversial, whereas it is generally accepted that the androgen production is increased in polycystic ovaries (JONES, 1964). Stein and Leventhal suggest that only biosynthesis of ovarian steroids is disturbed in the syndrome named after them, and that adrenocortical function is intact. The majority of other investigators, however, suspect that there is concomitant adrenocortical dysfunction in at least some of these women.

Few histological investigations have been performed on the adrenals, and these have never revealed adrenocortical hyperplasia in patients with polycystic ovaries. The absence of hyperplasia does not of course exclude increased production of adrenocortical androgens. Thus results with cortisone (see below) show that there may be adrenocortical hyperactivity in some cases of polycystic ovaries. These patients probably have so-called "borderline adrenal hyperplasia" (GOLD, 1958; ZENER, 1961).

It must not be forgotten, however, that corticoids can increase gonadotropin excretion (SHEARMAN, 1966) and thus act indirectly on the ovaries. 17-Hydroxycorticoids are occasionally found to be elevated and the excretion of 11-oxy-17 ketosteroids is rarely increased. This indicates involvement of the adrenal cortex (JONES, 1953; GREENBLATT, 1953).

It is also known that hypothalamic lesions induced in experimental animals to produce polycystic ovaries also cause hyperplasia of the adrenal cortex at the same time (BARRACLOUGH, 1961).

Particular attention has been devoted to the excretion of metabolites of C-19 and C-21 steroids in the hope of discovering their site of origin (MAUVAIS, 1962), but this has not been revealed. Excretion of 17-ketosteroids and 17-hydroxysteroids may be normal in polycystic disease of the ovaries or elevated in rare cases. In a statistical sample of 338 cases of polycystic ovaries confirmed surgically, 23% showed high values (GOLDZIEHER, 1963). Stein and Leventhal only count patients with normal 17-ketosteroid excretion as cases of the syndrome named after them. A normal 17-ketosteroid excretion does not exclude an elevated production of testosterone. A 100% increase in the formation of testosterone, e.g. from 3 to 6 mg daily, causes in increase of only 1.5 g in the 17-ketosteroid excretion, but 180 g testosterone secreted in a month can produce hirsutism in the female. The raised androsterone and etiocholanolone fraction in the presence of quantitatively normal 17-ketosteroid excretion shows that formation of androgens is raised in these cases of polycystic ovaries.

Pregnanediol excretion corresponds to preovulatory values. Pregnanetriol excretion is normal. In view of the normal pregnanetriol excretion in the Stein-Leventhal syndrome, it is hardly probable that the pregnanetriolone often excreted in increased amounts at the same time is of adrenocortical origin.

Since it has become possible to estimate the *level of testosterone* in the plasma precisely by means of double labeling with isotopes (RIONDEL, 1963), it has been shown that the average value of free testosterone in the plasma is three or four times higher than normal in patients with polycystic ovaries (FORCHIELLI, 1963; LOBOTSKY, 1964; DIGNAM, 1964; KORENMAN, 1965; LLOYD, 1966) (Table 52). This is consistent with the elevated excretion of testosterone glucuronide in the urine (PRUNTY, 1967).

Table 52. Plasma testosterone

Group of patients	Testosterone (μg/100 ml) mean ± SD	17-ketosteroids (mg/24 h) mean ± SD
"Normal" men	0.640 ± 0.20	
"Normal" women	0.036 ± 0.009	11.5 ± 3.5[a]
Women with polycystic ovaries and hirsutism	0.135 ± 0.128	10.4 ± 2.8[a]
Women with polycystic ovaries *without* hirsutism	0.110 ± 0.058	10.0 ± 3.8[a]

[a] LLOYD (1966).

The therapeutic results obtained by STEIN (1963) with the wedge resection in very carefully selected cases, have not been achieved by any other author. A series of laboratory investigations was developed in the hope of differentiating the cases likely to show a favorable response to wedge resection of the ovaries because of the ovarian origin of the androgens. These tests were intended to distinguish between androgens arising from the adrenal cortex and those originating in the ovary. Fractionation of the 17-ketosteroids shows an increase in the androsterone and etiocholanolone fraction in women with polycystic disease of the ovaries. This however yields no information about the site of origin of the androgens. *Dynamic function tests* for differentiation between adrenocortical and ovarian steroids have also proved disappointing. The results obtained vary widely and must be interpreted with the utmost caution. Only about 50% of patients with polycystic ovaries show no significant reduction in 17-ketosteroid excretion in the dexamethasone inhibition test. This may be because 17-ketosteroids arise outside the adrenals, or because there is a state of autonomy for the synthesis of adrenocortical androgens, as has been observed in certain forms of adrenal disorders (GALLAGHER, 1956–57; GOLDZIEHER, 1960). Estimation of the excretion of 17-ketosteroids and fractionations during adrenocortical inhibition with corticoids and simultaneous stimulation of the ovaries with gonadotropins (JAYLE, 1961; MAHESH, 1964) gives no definite results, and only demonstrates the wide variation associated with the Stein-Leventhal syndrome. Interference due to simultaneous inhibition and stimulation of the incretory organs (PERLOFF, 1963; SOHVAL, 1951) and the influence of reciprocal relations in the hypophyseal regulation of adrenal cortex and ovary greatly restrict the value of dynamic function tests. This is also applicable to investigations on the behavior of plasma testosterone under the action of dexamethasone and gonadotropin stimulation (LLOYD, 1966). As the adrenocortical production contributes 20 μg/100 ml to the testosterone in the plasma (PRUNTY, 1967), a pronounced fall in the plasma testosterone level due to dexamethasone indicates raised androgen synthesis in the adrenal cortex. This was found to be the case in three of the nine women with polycystic ovaries investigated by LLOYD (1966).

To summarize the numerous results: it appears possible that there is androgen hyperactivity of the adrenal cortex in some cases of polycystic disease of the ovaries, but there is still no proof of this.

Urinary estrogens and the rate of secretion of estrogens are usually within the normal ranges (BARLOW, 1967). Elevated excretion values have been described exceptionally (BROWN, 1963). It is possible that there are qualitative differences, in the form of reduced estriol excretion, from the normal ovary (BARLOW, 1967).

In keeping with the increased testosterone concentration in the plasma, the androgen/estrogen ratio, which is important for peripheral steroid activity, is also raised (MAHESH, 1962).

Very few results are yet available about the excretion of *gonadotropins* in cases of polycystic ovaries, and they allow no conclusions. Excretion of the total gonadotropins is in no way unusual (PRUNTY, 1967). The few studies in which excretion of FSH and that of LH are measured separately show irregular elevation of LH activity (INGERSOLL, 1959; KEETTEL, 1959; TAYMOR, 1963; KELLER, 1968) while FSH activity is unaltered (BUTT, 1967).

The impairment of estrogen synthesis from androgens in the polycystic ovary has also been disputed. A number of investigators have

observed normal biosynthesis of estrogens *in vitro*, a normal or even elevated estrogen content in the follicular fluid of polycystic ovaries, and a normal secretion rate (BARLOW, 1967). Other investigators suspect that estrogens originate mainly in plasma androgens, and are not secreted as such by the ovaries (MCDONALD, 1966).

The *diagnosis* of polycystic ovaries should be made by elimination, patients with Stein-Leventhal syndrome forming a subdivision (Table 53). The syndrome must be differentiated from the post-puberal form of the congenital adrenogenital syndrome and less frequently from Cushing's syndrome and virilizing tumors of the ovary and adrenal cortex. A raised 11-ketopregnanetriol excretion is pathognomonic of the congenital adrenogenital syndrome. A definite increase in the excretion of 17-ketosteroids indicates an androgen-producing tumor in the ovary or adrenal cortex. A DHEA fraction of over 50% is also indicative of the presence of an adrenal tumor.

Table 53. Syndrome with polycystic ovaries

Stein-Leventhal syndrome	
Sterility/anovulation/oligomenorrhea/amenorrhea/ functional bleeding/ hirsutism (virilization)/obesity	
One or both ovaries enlarged and polycystic (oysterlike)	Normal-sized polycystic ovaries hyperthecosis ovarii
17-ketosteroids and 17-OH-corticoids normal	17-ketosteroids and/or 17-OH corticoids slightly elevated
Increased excretion of Δ_5-3β steroids in the urine	
Ovulation not induced with cortisone	Ovulation induced with cortisone

The many women with so-called *simple or idiopathic hirsutism* must also be differentiated from women with polycystic disease of the ovaries and hirsutism. These women show no detectable disorder of ovarian and adrenocortical function. The cycle is only exceptionally disturbed, and the ovaries show no macroscopic or microscopic changes. The hirsutism is frequently a racial characteristic, and is found particularly in women of Mediterranean races.

The etiology of polycystic ovaries is still open to discussion. A primary disorder of the synthesis of ovarian steroids due to a genetic defect cannot be excluded. A genetic disturbance is suggested by the simultaneous occurrence of a deficit of 3β-hydroxy-steroid dehydrogenase in the adrenal cortex and polycystic ovary (AXELROD, 1965), the simultaneous occurrence of genital anomalies (CHOSSON, 1958), and a chromosomal mosaic (BISHUN, 1964, etc.). [The last factor has, however, not been confirmed by other investigators (BYRD, 1964).] Taking the therapeutic success of wedge resections and of treatment with clomifene or gonadotropins into consideration the current tendency is to evaluate the disturbed ovarian synthesis of steroids as secondary in nature due to impaired hypothalamo-hypophyseal regulation. This view is also supported by animal experiments in which polycystic ovaries or a state of continuous estrus is produced by damaging the "rhythmic" sexual center of the anterior hypothalamus with testosterone (BARRACLOUGH, 1961). Clinical observations in cases with hypothalamic lesions also support this (BARTUSKA, 1967).

The results available at present allow formulation of the following hypothesis on etiology and pathogenesis: some unknown factor causes a disturbance in the rhythmic release of FSH and LH by the anterior pituitary. The rhythmic LH spurt necessary for the release of ovulation in particular is absent. The causal agent can be a constitutional or emotional factor, an endogenous hormonal influence from the adrenal cortex or thyroid gland, or exogenous medicinal or mechanical influence acting on the hypothalamic center responsible for the rhythmic release of gonadotropins. This results in loss of the rhythmic release of gonadotropins, such as occurs in the male. This in turn leads to failure of terminal follicular maturation. Cysts are formed, numerous atresias with functional disturbance in steroid synthesis cause increased androgen production and thickening of the capsule, as SCOTT was able to show experimentally (1956). The capsular thickening, however, is in no way related to the failure of ovulation; removal of one ovary induces ovulation in the other polycystic ovary.

Treatment

Women are primarily forced to consult their physicians because of sterility, severe functional uterine bleeding, and less frequently because of oligomenorrhea or amenorrhea. Even in the absence of any desire for children, treatment of the cycle disorder is indicated because inadequate stimulation of the endometrium frequently leads to atypical hyperplasia (ZANDER, 1962) or even to carcinoma of the body of the uterus (ANDREWS, 1960; GOLDZIEHER, 1962).

Sterility and the possibility of endometrial disorders are not the only reasons for treating these women. Continuous increase in the production of androgens can lead to hirsutism and even to virilization, and the anabolic action of androgens may cause obesity.

The original form of treatment recommended by STEIN and LEVENTHAL (1935) was *wedge resection of the polycystic ovaries*. Very careful selection of patients and resection of an adequate portion of the ovary with hilar tissue are essential for therapeutic success. The final size of the treated ovary should be rather less than that of a normal ovary. The success is very variable and this is certainly partly connected with the choice of cases and the surgical procedure. Unfortunately there are still no criteria for preoperative assessment of the chances of success with the wedge resection (see above). Size of the ovaries and 17-ketosteroid excretion are not important (GOLDZIEHER, 1962; TAYMOR, 1963). STEIN (1963) achieved 95% ovulatory cycles and a conception rate of 80% in a total of 108 cases. These results, however, have not been obtained by any other investigator. It was deduced from a large group of collective statistics that the cycle becomes normalized in a mean of 80% of cases, the limits ranging between 6 and 95% (GOLDZIEHER, 1963) (Table 54). Pregnancy arose in 13 to 89%, average 63%, whereas hirsutism diminished in 16% of the women (range 0–18%). Our experience and that of others (GOLDZIEHER, 1962; TAYMOR, 1963), however, suggests that the curative effect of the wedge resection is only temporary. STEIN disagrees with this on the basis of his own experience (1964, 1967). There is at present no satisfactory explanation for the therapeutic effect of the wedge resection. The reduction in the ovarian tissue producing androgens has some effect. This was deduced from estimations of plasma testosterone before and after surgery (Table 55). It can be assumed, however, that the disturbed ovarian biosynthesis is only quantitatively and not qualitatively changed by the mechanical procedure (ZANDER, 1963).

Table 54. Published results of wedge resection performed in a total of 1079 cases (GOLDZIEHER, 1962)

Result	Prevalence (%)	
	Mean	Range
Regular cycles	80	6–95
Pregnancy	63	13–89
Lessened hirsutism	16	0–18

Table 55. Plasma testosterone (μg/100 ml) before and after ovarian wedge resection (4 patients) (LLOYD, 1966)

Before surgery	After surgery	
0.177	Day 1	0.058
	Day 6	0.098
	1 month	0.082
0.089	4 months	0.030
	18 months	0.037
0.077	16 months	0.081
	21 months	0.037
0.086	Day 1	0.039
	Day 6	0.047

Patent tubes and normal fertility in the husband are necessary requirements for wedge resection. In our opinion, wedge resection should not be performed prophylactically in view of later sterility and hirsutism, and particularly not in teenagers with polycystic ovaries (STEIN, 1964), since relapses are not uncommon and the desired pregnancy usually arises within a few months after surgery.

JONES, HOWARD, and LANGFORD were the first to report on the regulation of cycles with cortisone treatment in women with polycystic ovaries in 1953. Their result have since been confirmed by numerous investigators (GREENBLATT, 1953; SMITH, 1965). The results in cyclic regulation, incidence of pregnancy and hirsutism correspond to those with the wedge resection, even in cases where the 17-ketosteroids are not raised. Cortisone also produces a fall in plasma testosterone levels (LLOYD, 1966). As long as there are no contraindications (TB, gastric ulcer), treatment with cortisone should be tried before wedge resection is considered. We use 10 mg prednisone daily for long-term treatment over 4–6 months. Treatment is continued for 12 months after ovulation arises, and the patient is followed up after discontinuation of the drug to check whether ovulation is still occurring.

In recent times, treatment with clomifene has had a high degree of success in polycystic ovaries (KISTNER, 1961, 1967). About 75% of women with polycystic ovaries begin to ovulate after treatment with clomifene (JOHNSON, 1967). It is best to start with a low dose of 50 mg daily for 4 days because of the sensitivity of polycystic ovaries to gonadotropic stimulation. The therapeutic action is probably due to a direct effect on the synthesis of ovarian steroids as well as to hypothalamic regulation. Ovarian dysfunction recurs in most cases after withdrawal of clomifene. Clomifene is the treatment of choice but should only be used in women who want children, who have patent tubes and whose husbands are normally fertile.

Good results have also been achieved by treatment with gonadotropins (LUNENFELD, 1960; STAEMMLER, 1964; GEMZELL, 1965; JONES, 1965). Because the ovaries are increasingly sensitive to gonadotropin stimulation, the dosage must be adjusted cautiously and patients carefully monitored. It is known that lutein cysts can appear and grow rapidly, leading to rupture and massive intra-abdominal hemorrhage (VAN DE WIELE,

1965). The danger of multiple pregnancies is also recognized. Treatment with gonadotropins is only indicated in individual, carefully chosen cases where other forms of treatment have failed and where there is a wish for children, because of the dangers involved and the high costs (for treatment schedule, Fig. 59).

The cyclic use of oral estrogens and progestins is recommended for young unmarried patients with polycystic ovaries (GOLDZIEHER, 1967). This form of treatment inhibits ovarian and adrenocortical function, thus suppressing the increased androgenic production without causing a peripheral deficit of ovarian hormones. The use of an ovulostatic compound will become established for practical reasons. A marked decrease in the size of bilateral enlarged polycystic ovaries has been described with this form of treatment (GOLDZIEHER, 1967).

4. The Premenstrual Syndrome and Dysmenorrhea

a) The Premenstrual Syndrome

The premenstrual syndrome includes psychological and physical symptoms occurring cyclically in the premenstrual phase (FRANK, 1931).

Psychological symptoms arranged in order of frequency are: nervous tension, irritability, lability of mood, aggressiveness, restlessness or depression, scared moods, insecurity, tiredness (LAMB, 1953; BLEULER, 1954). Psychoses may exceptionally arise (BLEULER, 1954; KROGER, 1962). Psychological symptoms may be associated with disturbances of partiality drives: abnormal hunger or thirst, insomnia, increased libido (BLEULER, 1954). The disturbance in the psychological equilibrium is reflected by the increase in female crimes during the premenstrual period (MORTON, 1953; DALTON, 1964) and by the high frequency of suicide, accidents and illnesses during the second half of the cycle (MCKINNON, 1966).

The social significance of the premenstrual syndrome is greater than the purely medical problem since psychological premenstrual disturbances are more frequent and severe than the physical symptoms.

Physical symptoms in order of frequency (MORTON, 1953) are: mastodynia (70%), abdominal symptoms (approximately 50%) such as distension in the abdomen, congestive states in the small pelvis, edema (approximately 45%) of the hands, feet, and face, headaches (approximately 30%), sometimes in the form of migraine attacks. Premenstrual water retention up to 4 liters with development of edema (THORN, 1938; GREENHILL, 1941; HAUSER, 1959; BRUCE, 1962; ROGERS, 1963) is the only pathognomonic physical symptom. Allergic features such as urticaria, vasomotor rhinitis and asthma occasionally arise cyclically in the premenstruum (DORMAT, 1957).

The data on the *frequency of the syndrome* vary widely depending on the evaluation of symptoms and the group of patients investigated. EICHNER (1959) found the syndrome in 70% of nurses, but less than 7% considered treatment necessary for their symptoms. According to ISRAEL (1967), the syndrome arises in 75% of women. It was present without exception in one group of adolescents studied, but treatment appeared necessary in only 2% (GALLAGHER, 1960).

The pathogenesis is under investigation. Almost all authors agree that *psychological factors* are involved, since the syndrome is frequently encountered in women with neurotic, psycholabile and psychopathic personalities (WILLIAMS, 1952; LAMB, 1953; REES, 1953; BLEULER, 1954; APPLEBY, 1960). In contrast to the situation in dysmenorrhea, there is no basic actual situation of conflict, but rather a latent neurotic attidude due to conflicts in life (KROGER, 1962). The interrelationship of mind and body is summarized by BLEULER (1954): "Premenstrual tension is an emotional shock which arises because of different hormonal, metabolic and physiological disturbances to the state of equilibrium if the psychological development of the woman is predisposed to this".

Metabolic disorders such as hypoproteinemia (MENZER-BENARON, 1963), elevation of blood sugar level (HAUSER, 1959), and hypoglycemia (MORTON, 1953) have been described.

The observation that the syndrome only arises in ovulatory cycles leads to the assumption that hormonal factors have some part in the etiology (GREENE, 1953). Quantitative hormonal investigations in large comparative series have shown, in contrast to earlier theories, that the excretion of estrogens and progesterone lies in the same range for women with the premenstrual syndrome as for other women, and that there is no significant connection with symptoms (LAMB, 1963; MENZER-BENARON, 1963; PRILL, 1963). An increased ergotropic-sympathetic reactive state with hypersensitivity to stimuli (KROGER, 1962; ARTNER, 1965) released by progesterone possibly plays some part. Corticoids may also be elevated (MENZER-BENARON, 1963).

According to some authors, premenstrual water retention has an important effect in the pathogenesis of the premenstrual syndrome (THORN, 1938, 1957; GREENHILL, 1941; FREED, 1945). The edema is thought to be produced

by secondary aldosteronism (STREETEN, 1960), aldosterone secretion being increased by progesterone and psychological states of tension (LAMSON, 1956; VENNING, 1957). This hypothesis is supported by the observation that aldosterone excretion is increased during the premenstrual syndrome, that sodium diuresis is decreased (CIMBERLE, 1961), and that administration of additional progesterone can raise sodium excretion after ovulation (JENKINS, 1961; LANDAU, 1958, 1961). Adiuretin is also considered to be of pathogenic importance in the development of the premenstrual syndrome. Vasopressin given intramuscularly during the luteal phase is alleged to be capable of inducing the premenstrual syndrome (BICKERS, 1952). Elevated adiuretin excretion has been reported during the premenstrual syndrome (ZUSPAN, 1958).

The importance of a "menotoxin" in the development of the premenstrual syndrome (MACHT, 1949; SMITH, 1950) is no longer taken seriously.

Treatment

Treatment with drugs and also psychotherapy alone can be successful. Discouragement from overestimation of the symptoms is important (ROEMER, 1969). The best results are obtained with combined medical and psychological treatment (REES, 1953; KROGER, 1962). Combined preparations containing a diuretic, a progestagen, and a tranquilizer are thought to be effective (BARFIELD, 1962). We have noticed an improvement in some cases treated with a combination of an antihistamine and a bromtheophylline preparation (Donasil). A low-salt, protein-rich diet has a supportive effect (MENZER-BENARON, 1963).

Only a few severe cases require psychoanalysis (KROGER, 1962).

b) Dysmenorrhea

Dysmenorrhea or algomenorrhea signifies either continual or more frequently cramp-like pains in the lower abdomen in association with menstruation. They sometimes begin shortly before menstrual bleeding, but usually with the onset and rarely after the bleeding. The pains may be associated with nausea and vomiting.

The symptoms usually last from a few hours to one day. They seldom persist for more than two days, and only exceptionally throughout menstruation. The pain usually reaches its maximum shortly after the onset of bleeding. Pains due to endometriosis are most severe premenstrually and decline with the onset of bleeding.

Since dysmenorrhea is not an illness but merely a symptom, the underlying illness must be searched for.

Two types of dysmenorrhea are differentiated: *dysmenorrhea without anatomical substrate* (functional, idiopathic, intrinsic, essential) and *dysmenorrhea with anatomical substrate* (organic, acquired, extrinsic, symptomatic). In German terminology, *primary dysmenorrhea* arising with menarche or shortly after menarche is also distinguished from *secondary dysmenorrhea*, which arises in later years. In the English speaking areas, primary and secondary dysmenorrhea are identical to functional and acquired dysmenorrhea.

The data on the *frequency* of dysmenorrhea vary widely since it is a subjective symptom (LOWE, 1951). In addition, it varies with the population group (DAVIS, 1938; DOSTER, 1961) and is dependent on whether mere malaise or inability to work is counted. Severe pains regularly confining the women concerned to bed were found in 3–15% of women in different professional categories (DOSTER, 1961), and 10% of school girls in high school are periodically absent because of dysmenorrhea (HEALD, 1957; GALLAGHER, 1960; GOLUB, 1963). In DOSTER's study 25% were absent once or repeatedly (1961). It has been calculated that 140 million working hours are lost yearly in USA because of dysmenorrhea (KISTNER, 1964). It is therefore an important social problem.

Dysmenorrhea can occur at any age. It is encountered most frequently during the third decade, with a maximum in the 25th year (FLUHMANN, 1963). A large number of organic changes claimed to be the cause of dysmenorrhea can be found in the literature. With a few exceptions there is no evidence of a causal relation.

Endometriosis is the first organic cause to be considered. It can cause the uterus to become fixed and retroflexed. Dysmenorrhea is occasionally produced by acquired stenosis after cauterization or operation of the portio.

A mobile, retroflexed uterus, fibroids, malformations in the absence of cervical or vaginal obstruction, primary stenosis of the internal and external os uteri are no more frequent in women with dysmenorrhea than in women without symptoms (KÖNIG, 1959; JAMES, 1963). Nor is there any causal connection between dysmenorrhea and uterine hypoplasia, which is diagnosed much too often (JEFFCOATE, 1945; KÖNIG, 1959; LUKAS, 1965). The same applies to chronic inflammatory processes in the pelvis.

Organic changes seldom cause dysmenorrheic complaints, whereas psychological factors play an important part in the development of dysmenorrhea (HERSCHBERG, 1960; PRILL, 1964).

According to our experience and that of other authors (HAUSER, 1959; LUKAS, 1963; ROEMER, 1969) dysmenorrhea is due to psychological factors in 90% of cases. The following psychological forces (living relations) play a considerable part (STURGIS, 1962; ROEMER, 1969): loss of domestic security, conflict over own femininity, dependence, hostility, feelings of guilt towards mother or sister (family rivalry), possibly identification with the mother who also suffers from dysmenorrhea (LOMER, 1899) ("familial dysmenorrhea" after A. MAYER, 1925), ambivalence between the subconscious wish to remain a little girl and the conscious drive to become grown up, fear of awakening sexuality, fear of pregnancy, fear of sterility, etc. Dysmenorrhea is thought frequently to be an expression of a demonstrative aggressive protest and resistence (ROEMER, 1969). On the other hand, the association of bleeding and pain is almost archetypically implanted (PRILL, 1961). Dysmenorrhea frequently arises in leptosomatic-asthenic, schizoid types who have difficulty in forming social contacts, and in dysplastic women (infantile, hypoplastic, dystrophic-adipose types). Consideration of the constitution shows that it is not the genital hypoplasia but the psychological immaturity associated with it that is of paramount importance. Psychosexual retardation predisposes to neurotic reactions to age-dependent stress (KRETSCHMER, 1952).

Various mechanisms have been discussed for the pathogenesis of dysmenorrheic pains. The observation that they arise almost exclusively with ovulatory cycles (WILSON, 1940) leads to the assumption that an endocrine factor is the releasing cause. The so-called "endocrine imbalance" has, however, not been proved so far (FLUHMANN, 1938). On the other hand, the rapid premenstrual fall of estrogens, and perhaps also of gestagens, leads to a sympathetic-adrenergic reactive state (Fig. 32). This reduces the stimulus threshold, converting otherwise below-threshold stimuli to stimuli above the threshold (HAMAN, 1944) with pathologic production of pain. The cycle-dependent physical disposition thus favors the development of pains in psychogenic dysmenorrhea, but is merely a predisposing factor. The response of smooth muscle to sympathetic-adrenergic stimuli is increased at the same time (WALTHARD, 1937). The hypothesis that this releases stronger and more frequent dysrhythmic contractions (MOIR, 1936; BICKERS, 1941; WOODBURY, 1947; MILLER, 1953) of the uterine musculature, or a functional sphincter is insufficiently relaxed in the region of the isthmus (YOUSSEL, 1958; MANN, 1961) or vasoconstriction of uterine vessels leads to ischemic pains analogous to conditions in the spiral arteries of the endometrium (MOIR, 1936; WOODBURY, 1947; PRILL, 1962) are plausible, but still purely speculative. More recently a so-called "menstrual-stimulant factor" has been discussed (CLITHERTOE, 1961). This factor has been isolated from menstrual fluid, and it was possible to induce uterine contractions with it (EGLINTON, 1963). It is claimed to be absorbed in increased amounts in dysmenorrhea.

Continuous *premenstrual* pains arising in endometriosis are probably due to tension of the capsule produced by premenstrual vascular congestion.

The colic-like pains in so-called *dysmenorrhea membranacea* are thought to be caused by rejection of incompletely decomposed endometrium resulting from an enzymatic disorder in the endometrium (GREENBLATT, 1954). This however is contradicted by the observation that decidual saccules are rejected painlessly in an ectopic pregnancy.

Diagnosis and Treatment

Since dysmenorrhea is a symptom and not an illness, the cause must first be detected. We adopt the following system: the uncommon organic causes are first detected or excluded by case history and gynecological examination. This, however, is not always absolutely certain. If there is no apparent organic cause, a thorough psychiatric case history must be taken. The psychological noxa can usually be found in this way. In a few cases, and according to other authors in 50% of cases (LUKAS, 1963), a cure is achieved with a brief course of psychotherapy, but it must also be remembered that 23% of cases of dysmenorrhea in young women are cured spontaneously (GOLUB, 1963). In our experience, treatment with analgetics or spasmolytics is required in the majority of women with dysmenorrhea of average severity. This must be supported by psychohygienic measures: sufficient sleep, physical exercise, regulation of bowel action, sensible balance between work and leisure. It is of interest that the most varied types of treatment produce a "cure" in an average of 80% of cases. This is also indicative of the pathogenesis. It can be deduced from this that treatment has a placebo effect, i.e. psychological factors play a dominant role.

In cases of severe dysmenorrhea, inhibition of ovulation by one of the hormonal contraceptives in use practically always results in painless withdrawal bleeding, or at the most with very little pain. The symptoms do however, recur within 2–3 months after discontinuation of ovulostatic. If these women want children the

whole situation must be reexamined with a view to simple (COTTE, 1937), or extensive (HELD, 1943) sympathectomy. After an operation of this type, birth occurs with less pain and more quickly. A birth sometimes leads to the disappearance of dysmenorrhea, but this rule is not very reliable.

The cause of the therapeutic success of a birth is not clear. It was previously presumed that this was due to pressure atrophy of Frankenhäuser's ganglia caused by the advancing head. This, however, now appears unlikely, since symptoms cease almost as frequently even after Cesarean section.

H. The Sterile Marriage

Investigations by various authors (TIETZE, 1950; BARNS, 1953; WHITELAW, 1960) have shown that over 50% of women become pregnant in the first month of regular intercourse without the use of any contraceptive, two-thirds within the first three months, rather more than three-quarters during the first six months and 80–90% within the first year (Fig. 66). Inquiries among soldiers' wives yielded similar results (NOTTEBOHM, 1948). Only one in 20 of the remaining women conceived during the 2nd year. SOUTHAM (1960) found somewhat lower figures, but also showed that only 8% of first pregnancies occurred during the second year of marriage.

Thus, a *marriage* can be described as *sterile* when pregnancy fails to occur after a year of regular intercourse without contraceptive precautions. Other authors (STÖCKEL, 1947; MARTIUS, 1964) do not apply the term sterility until a woman has failed to become pregnant after two years of regular married life where there is a desire for children. This definition is incorrect in both theory and practice. Precious time is unnecessarily lost in waiting, especially in women over 30, who are frequently found in groups of sterile women.

Primary sterility is present when there is no proof of a pregnancy ever occurring, and *secondary sterility* when after having given birth to one or more viable fetuses a woman does not become pregnant again within one year of regular unprotected intercourse.

In contrast to the English terminology, a distinction is made between infertility and sterility in German.

Infertility implies the inability to carry the pregnancy until the fetus is viable. It too can be *primary* or *secondary*. *Habitual abortion* is spoken of when three or more consecutive pregnancies have all failed to reach the stage of fetal viability.

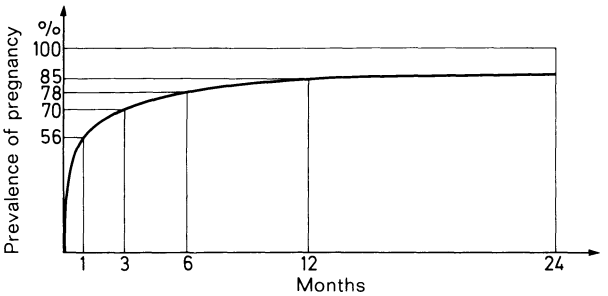


Fig. 66. Prevalence of pregnancy as a function of the period of regular intercourse without contraception. (After WHITELAW, 1960)

1. Frequency

In Europe and U.S.A. 10–15% of marriages are childless. For obvious reasons, the relevant statistics do not distinguish between wanted and unwanted sterility. Figures based on the Swiss population in 1960 allow the conclusion that deliberate childlessness in the early years of marriage plays an important part in this country (Table 56).

Table 56. Number of childless marriages in Switzerland (1960)

Total number of marriages		Number of childless marriages
Of up to 1 year's duration	42942	20317 ≙ 50% (48.0%)
Of 1–2 years' duration	42916	14715 ≙ 34% (34.3%)
Of 2–5 years' duration	40088	8979 ≙ 25% (24.9%)
Of 5–10 years' duration	35522	7057 ≙ 20% (19.8%)
Of 10–20 years' duration	26115	4549 ≙ 17% (17.4%)
Of 20 or more years' duration	475735	70383 ≙ 15% (14.8%)

There is a considerable decline in the number of childless marriages between the 2nd and 5th years of marriage. However, about 22% of marriages are still childless even after at least 5 years. Few women conceive after the 5th year of marriage, and 14.8% of marriages are still sterile after 20 years. In 1941 11.5% of marriages of at least 20 years' duration were childless in Switzerland. So an increased number of childless marriages has been recorded in Switzerland within the last two decades.

The matrimonial difficulties arising from childlessness are multiple. Examination, treatment and counselling of childless married couples are an important part of a doctor's work.

2. Causes

Sterility is a symptom which can be caused by numerous etiological factors. Two or more

etiological factors were present in sterile marriages in 10.2% of our patients (GRUBER, 1973), and in as many as 60% of BALIN's cases (1967). MEAKER (1940) and HAMBLEN (1942), report 2.23 or 4.75 factors per married couple respectively. Prognosis and treatment are only possible after systematic investigation and careful interpretation of the results. However, in no other medical field are indifference, therapeutic nihilism and polypragmasy of the doctor and lack of cooperation, especially from the husband, so pronounced.

Marriage represents a biological unit, and it is therefore logical that investigation of sterility should involve both partners. Systematic investigation according to a set plan is essential for prognosis and therapy. The fertility of a marriage is the sum of the fertility of both partners. Reduced fertility in one partner may result in a sterile marriage as defined above, despite the fact that the other partner is normally fertile. Women who are more responsive to therapy and those who become pregnant after the course of a year without any treatment are placed in the group of subfertile or sterile marriages. Subfertility in one partner can be compensated for by high fertility in the other. The prognosis is much more unfavorable when both partners are subfertile.

Not only female sterility factors but also the male factor is of great practical importance. The data on the percentage involvement of single factors in a group of sterile marriages vary from author to author for different reasons: the "normal values" used in the individual methods of investigation differ since they are empiric, arbitrarily fixed standards and not absolute values. The danger of overestimating a single result, especially if based on only one case, is particularly great in the field of sterility (cf. Section on tubal factor or quality of sperm). Apart from this, the groups investigated differ in their socio-economic structure, age and geographical environment. Thus, the frequency of genital tuberculosis ranges between 0.9%–17.4% (HELD, 1947; CABALLERO, 1966) according to the region. It is for example, of greater importance as a cause of sterility in Israel and Scotland, and especially in Sweden, Spain and India, than in for example Australia and U.S.A. Endometriosis and hormonal factors are more frequently encountered in higher social classes than in lower income groups. This is due partly to the older age at marriage in the former classes. Endometriosis is found more frequently in U.S.A. than in Europe. Inflammatory genital changes are more common in the lower social groups. In addition, the fertility of a woman is greatly dependent on her age (DIDDLE, 1947; WYLE, 1957). Fertility is at its maximum between 15 and 25 years, decreases rapidly by half by the age of 30, and is only $\frac{1}{3}$ to $\frac{1}{6}$ of its original level at 35 (MÜNZNER, 1934). Only 3 of 100 childless women have a child after the age of 40 (Table 57, Fig. 67). The decline in female fertility corresponds roughly to the reduction in the number of primordial follicles in the ovaries (PEARL, 1939; Table 1). Even 100 years ago, DUNCAN (1866) recorded that 90% of Scottish women married between the ages of 20 and 24 became pregnant within 2 years, whereas this was only true of 63% of women married between 30 and 34 and of 15% of those married between 40 and 45.

Table 57. Declining fertility with advancing age in woman (KOPPEN, 1951)

Marriage at	No. of children within			
	1 year	2 years	3 years	4 years
18 years	0.49	0.76	0.96	1.16
35 years	0.14	0.27	0.48	0.62

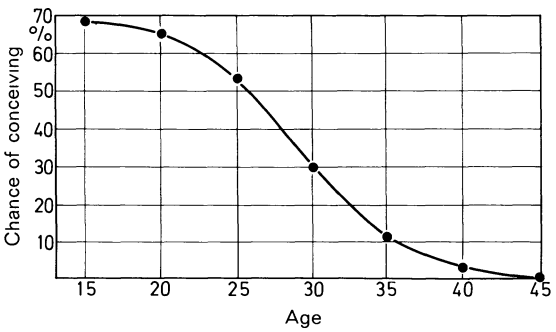


Fig. 67. Female fertility related to age. (After MÜNZNER, 1934)

A comparison of different statistics is further handicapped by different classification principles (ISRAEL, 1967; KISTNER, 1964; BICKENBACH, 1967). In general, involvement of male factors in sterility is 30–40% (GUERRERO, 1964), that of female factors 35–45%, and that of combined factors 10–20% (SLOAN, 1964; GRUBER, 1973). According to most statistics, about 30% of the causes of female sterility are due to changes in the tubes (BALIN, 1967; BICKENBACH, 1967; GRUBER, 1973). GRUBER (1973) investigated 106 cases of primary sterility and the percentage distribution of the main female sterility factors is presented in Fig. 68. In 11.7% of cases both partners prove to have at least one sterility factor. Other investigators have arrived at similar figures for the frequency of individual female sterility factors.

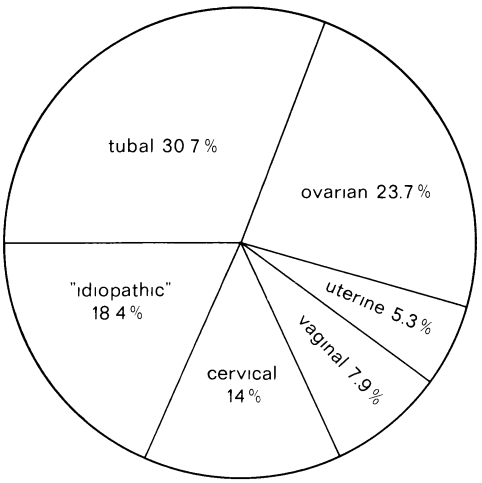


Fig. 68. Percentage proportions of female sterility factors concerned in 106 cases of primary sterility. (After GRUBER, 1971)

The cause of sterility cannot be determined in 10–20% of all sterile couples (GRUBER, 1972). In such cases so-called *idiopathic sterility* is present. This is not surprising since we are still only poorly informed about most of the processes occurring between insemination and impregnation and in the 6–7 days between impregnation and implantation. The diagnosis "idiopathic sterility" should only be made after prolonged and thorough investigation and observation by experienced investigators, and after culdoscopy or laparascopy has also yielded normal findings. In some cases, both partners are subfertile. In others, there may be an immunological incompatibility between sperm and female organism as is suggested by the observation that remarriage with other partners has often resulted in fertility of both partners of the original marriage. According to BEHRMAN (1965), immunological incompetence of this kind is present in 20% of cases of so-called idiopathic sterility. As has been shown by investigations by HERTIG, ROCK and ADAMS (1956), unviable fertilized ova compose a large proportion of so-called reproductive wastage. In this study, 13 (or 38%) of 34 fertilized human ova were found to be abnormal in the first 17 days. Since the rate of spontaneous abortion is 8.2% (JAVERT, 1957), about 30% of impregnated ova are lost without the woman involved becoming aware of this. An indefinite number of these women are classified in the group of idiopathic sterility. If the cases of extra-uterine pregnancy and intra-uterine deaths are also taken into account, so-called reproductive wastage involves over 40% of all ova, not including legal and illegal abortions.

Normal female fertility is dependent on numerous factors. Only a limited number of these can be investigated by methods of investigation currently available. If they all give normal results fertility is probably normal, but the only proof of this is conception and the birth of a living child.

Present-day knowledge of the physiopathology of reproduction and the techniques available for investigation suggest that in practice 6 factors are essential for normal female fertility. These are the so-called vaginal, cervical, uterine, tubal, endocrine, and psychogenic factors.

The most frequent causes are briefly mentioned below. The reader is referred to the appropriate specialized literature for more detailed information (HOTCHKINS, 1944; PALMER, 1950; BERNHARD, 1947; SIMMONS, 1954; BUXTON, 1958; TYLER, 1961; WILLIAMS, 1967; STALLWORTHY, 1966; WESTIN, 1966; THOMAS, 1966; BICKENBACH, 1967; ISRAEL, 1967).

a) The Vaginal Factor

Severe anatomical changes in the vagina alone, such as aplasia, do not cause sterility, since they are associated with severe obstructive malformation of the uterus (so-called Mayer-Rokitansky-Küster syndrome) (HAUSER, 1961). Severe congenital stenosis of the upper third of the vagina very occasionally leads to sterility, but this can be corrected by a vaginal plastic operation.

Severe vaginitis can impair conception due to the presence of numerous leukocytes which damage the sperms enzymatically, or phagocytize them. This is especially applicable to vaginitis and cervicitis caused by trichomonads.

b) The Cervix Factor
(SÉGUY, 1933; HUSSLEIN, 1953; ANTOINE, 1957)

The quality and quantity of cervical mucus are essential to sperm penetration and capacitation (CHANG, 1955). Both are affected by hormonal, anatomic and bacterial factors (p. 538).

Acute or chronic cervicitis can cause sterility (SOBRERO, 1962). Chronic cervicitis may be due to erosions, ectopia or cancerous changes in the portio of the cervix. There may be antibodies in cervical mucus which act cytotoxically against the sperm of the husband. These antibodies can sometimes be demonstrated in the serum, and may lead to the so-called *allergic sterility* in women in whom the other fertility factors are not found to be pathologic (PARISH, 1968).

It is to be expected that in the next few years a series of further factors connected with reproduction will be obtained in the immunological field (KATSH, 1967). This would make the group of patients with idiopathic sterility even smaller.

The Sims-Huhner test, the fern test and the spinnbarkeit of cervical mucus are used for the assessment of the so-called cervix factor (p. 538). The crossed invasion test *in vitro* (MILLER-KURZROCK, 1932) for the investigation of a possible intolerance between sperm and cervical mucus is practically difficult and is therefore of little significance.

c) The Uterine Factor

Traumatic amenorrhea (p. 593) with or without uterine adhesions or synechia (Asherman's syndrome, métrose de réceptivité) can cause sterility. It is seldom seen in this country, but appears to be more common in Italy (MAGGIONI, 1963; STALLWORTHY, 1966).

Inadequate secretory transformation of the endometrium, bringing about a developmental retardation of at least 3 days, is seen in 3–10% of sterile women (GILLAM, 1955). Insufficient luteal conversion of the endometrium may be suspected when the hyperthermal phase lasts for less than 10 days, or when the temperature rise following ovulation occurs slowly in stages (DÖRING, 1952; OBER, 1952). The incidence of tuberculous endometritis is dependent on the geographical region and varies between 0.69–17.4% (CABALLERO, 1966). HELD (1945) found it in 1.6% of sterile women with patent tubes. It is not an absolute obstacle to pregnancy, since there are known cases of pregnancy in women with tuberculous endometritis (FISHER, 1962; STALLWORTHY, 1963). The prognosis depends on the state of the tubes at the onset of therapy.

The incidence of pregnancy varies from approximately 1% (SCHAEFER, 1964) to 15% (SUTHERLAND, 1966), 20% (SNAITH, 1962; RYDEN, 1966) to 40% respectively (SCHAEFER, 1964) if the tubes are patent at the onset of treatment, but the number of tubal pregnancies and abortions is greatly elevated in these women. STALLWORTHY (1963) observed 5 tubal pregnancies and 3 abortions in 17 pregnancies after tuberculous endometritis. SUTHERLAND (1966) described 25 pregnancies in 165 sterile women. Only 6 live children were born, and the remaining pregnancies aborted (8) or were ectopic (11).

The diagnosis of uterine hypoplasia is made much too frequently. The size of the uterus is no measurement of functional capacity.

Hypoplasia responsible for sterility is associated with delayed menarche and retarded, inadequate development of the secondary sexual characteristics. The small, infantile uterus is usually anteverted and anteflexed at an acute angle and is displaced towards the left; the cervix is as long as or longer than the body, and the length of the uterine sound is 6 cm or less. A uterine malformation can result in infertility but not in sterility. Sterility is caused only by uterine aplasia or the uterus solidus in the Mayer-Rokitansky-Küster syndrome (p. 588). The mobile retroflexed uterus itself does not lead to sterility. The fixed retroflexed uterus indicates tubular sterility with endometriosis or a state following pelvic peritonitis.

Fibroids are usually only a concomitant factor and are not involved in the etiology. In exceptional cases they may lead to interstitial closure of the tubes when they are in the relevant position (intramural, and less commonly submucosal fibroids), or they may cause tubal distortion, impairing the mechanism for collection of the ovum (subserous fibroid). On the other hand they are responsible for 20–25% of cases of infertility (ISRAEL, 1967; BUXTON, 1958).

The uterine factor is investigated by means of the endometrium biopsy and hysterosalpingography.

d) The Tubal Factor

Complete bilateral occlusion of the tubes following salpingitis, pelvic peritonitis or endometriosis is one of the most common causes of female sterility. In contrast, so-called partial tubal occlusion is seldom a cause of sterility. This type of occlusion is usually due to functional disturbances. This was deduced from RUBIN'S investigations (1932). He observed practically the same number of pregnancies in women with spastic and stenosed tubes and those with normal tubal patency.

Salpingitis now arises most often after abortion, particularly after criminal abortions, and less commonly with perityphlitis and during the puerperium. The pathogens are mainly E. coli, streptococci and staphylococci. Inflammation of the tubes due to gonorrhea and tuberculosis is quite rare in this country. It is questionable, however, whether gonorrhea has not in fact become more common, but can no longer be demonstrated due to the extensive use of antibiotics. The frequency of tuberculous salpingitis is closely related to the type of patients examined and to geographical factors (RIPPMANN, 1966). SHARMAN (1952) calculated that in Scotland over 25% of tubal occlusions

with primary sterility were due to tuberculosis. About 10% of patients suffering from pulmonary tuberculosis later develop genital tuberculosis (HELD, 1945). In women over 35 endometriosis plays some part in causing tubal sterility (PHILIPP, 1937; RIVA, 1961).

The methods – pertubation, hysterosalpingography, and laparoscopy/culdoscopy – available for investigation of the tubal factor can at the most show whether the tubes are patent or not (p. 627). They provide no information about the physiology of the tubes, which is of the utmost importance for maturation of sperms (so-called capacitation) (CHANG, 1955; NOYES, 1960). Sperms are not capable of fertilizing an ovum until they have been in the female genital tract, particularly in the tube, for 4–6 hours. We do not know how this "sperm maturation" comes about; it may be due to some enzyme activation.

The culdoscopy/laparoscopy alone allows an assessment of the mechanism of collection of the ovum, i.e. the spatial and functional relation between ovary and fimbrial funnel, which is quite frequently impaired by inflammatory and endometriotic changes (KISTNER, 1962). Only in 20–25% of sterile women in whom all other fertility factors are normal are the culdoscopic findings physiological (KELLY, 1956).

e) The Endocrine Factor

There are numerous conditions resulting in endocrine factors which can lead to disorders in the cyclic course of maturation of the ova and ovulation and the formation of estrogens and progesterone. These include: dysfunction and tumors of the hypothalamic-hypophyseal system, such as Simmonds' syndrome and Sheehan's syndrome, ovarian dysfunction, such as polycystic ovaries (p. 609), hyper- and hypothyroidism (Chap. VI), dysfunction or tumors of the adrenal cortex, such as socalled "borderline adrenal hyperplasia" (p. 612), the adrenogenital syndrome, Addison's disease and Cushing's syndrome. Diabetes mellitus and relative insufficiency of the corpus luteum also belong to this group. The last condition is indicated by a stepwise increase in temperature due to the delayed action of progesterone, and by a shortened hyperthermal phase, which lasts less than 10 days, in contrast to the usual 12–13 days (DÖRING, 1952; OBER, 1952). Such cycles are infertile. These cycles are usually shorter than normal and their incidence varies with age (Table 58). They are more common in women with sterile marriages, and depending on the author have been observed in 15–33% of sterile patients (PALMER, 1949, 1953; RAUSCHER, 1959; GRANT, 1959; DÖRING, 1960).

Table 58. Prevalence of anovulatory cycles as a function of age (COLLETT, 1954)

Age	No. of women	Cycles	Anovulatory cyles	
			No.	%
17–18	59	81	25	30.8
20–24	33	112	11	9.8
25–29	21	45	2	4.4
30–34	11	23	0	0.0
35–39	13	33	4	12.1
40–50	11	33	5	15.1
Total	148	327	47	14.3

Endocrine disorders cause amenorrhea (p. 585), oligomenorrhea or anovulation (p. 603) with dysfunctional hemorrhage. Inadequate secretory transformation of the endometrium is also produced. The diagnostic procedure is discussed in the appropriate chapters. Extensive differentiation is obtained from the basal body temperature.

f) The Psychogenic Factor

Psychogenic factors can interfere decisively with reproductive processes centrally by acting via the limbic system in hypothalamic centers (p. 555). Hypothalamic amenorrhea is an example of this [influence of psychological factors on the quality of sperms (Chap. IX, p. 483)]. However it is very seldom possible to differentiate psychological influences from those of organic nature. The diagnosis is made by elimination: comparative series are not available. A few striking cases support the importance of psychological factors, but this is also contradicted by just as many observations. The mode of action is frequently obscure or controversial. Thus it is uncertain to what extent tubal spasm or disturbed uterine motility can influence sperm transport.

Experience has shown that referral of the patient to the psychiatrist is essential in very few cases. Sympathetic listening and discussion of the problems of the married couple are therapeutically effective in many cases.

3. Methods of Investigation (Table 59)

Investigation of a sterile married couple must be carried out systematically according to a fixed plan. The diagnostic procedure is presented in Table 59. We are of the opinion that the investigation must be done separately for each partner. Only in this manner is it possible to obtain a complete history and a reliable impression of the attitude of both partners towards the possibility of having a child.

After completion of the investigations the results should be discussed conclusively with both partners together, and a possible plan of treatment should be decided on. If treatment is hopeless, the reasons must be explained to the couple. This will prevent further useless investigations and enable the couple to become adjusted to the idea of a possible adoption.

Table 59. Examination of the woman

A. Case history (in husband's absence):	
1. General:	Venereal diseases, tuberculosis, hypo- or hyperthyroidism, diabetes mellitus, alcohol, nicotine
2. Gynecological:	Menarche, menstrual cycle (interval, midcycle bleeding, duration of bleeding, mittelschmerz, intermenstrual discharge), induced abortions, adnexitis, gynecological operations, intercourse (frequency, time, technique, orgasm)
3. Psychological:	Reason for wanting children, attitude to husband, marriage, children, husband's parents, interests
B. Status praesens	
1. General:	General condition, hirsutism, virilization, blood pressure, ESR, blood picture, serologic lues reaction, urinalysis (albumin, sugar). If indicated BMR, PBI
2. Gynecological:	Hypoplasia, rigid hymen, vaginitis, cervical erosion, cervicitis, lacerations, macroscopic anatomy of genital organs: position, size, form, and motility of uterus, tubes and ovaries (tumors, adnexitis, parametritis) Shorr and Papanicolaou smears, detailed colposcopy, bacteriological examination of vaginal smear (trichomonas and monilia)
C. Special tests	
1. Basal temperature curve	
2. Endometrial biopsy ± excretion of pregnandiol in urine	
3. Sims-Huhner test, spinnbarkeit, fern-leaf test	
4. Pertubation ± hysterosalpingography	
5. Culdoscopy/Laparoscopy	
6. Hormonal examinations: 17-ketosteroids, 11-OH steroids, pregnandiol, HPG, PBI	

Fertility in a woman is dependent on the anatomy, function and biology of her reproductive system. Various methods are available for examination.

Measurement of the *basal body temperature* or the *temperature on waking* (GILES, 1901; VAN DE VELDE, 1905; RUBENSTEIN, 1938; VOLLMANN, 1940) permits the diagnosis of follicular rupture and formation of the corpus luteum on the basis of the thermogenic action of progesterone (Fig. 69). It is of practical significance in the treatment of the sterile couple for a rough determination of the time of ovulation (DÖRING, 1957). The fact that a corpus luteum can also occasionally be formed in the absence of ovulation slightly limits its value as a method of diagnosing ovulation.

The temperature can be measured orally or rectally. The oral temperature is 0.1–0.2 deg C lower than, but parallel to the rectal temperature (HARTMAN, 1962). The temperature must be taken in the morning for 5 minutes after at least 6 hours' night rest, and if possible always at the same time immediately after waking (temperature on waking). Since the temperature differences are very small, it is important to prepare the thermometer the previous evening and always to use the same instrument. An ordinary thermometer can be used for this purpose, but special thermometers are also available commercially, on which the scale between 35° and 38° is extended, making the reading easier (e.g. Cyclotest thermometer). There is an average rise in temperature of 0.4–0.6 degC 14 ± 2 days before the onset of menstruation (ROCK, 1949), most frequently 1–2 days after rupture of the follicle. This rise may take the form of an increase within 1–2 days or of increments over 3–7 days. In one third of women (PLOTZ, 1950) there is a fall in temperature before the rise. The temperature falls again within one day before the onset

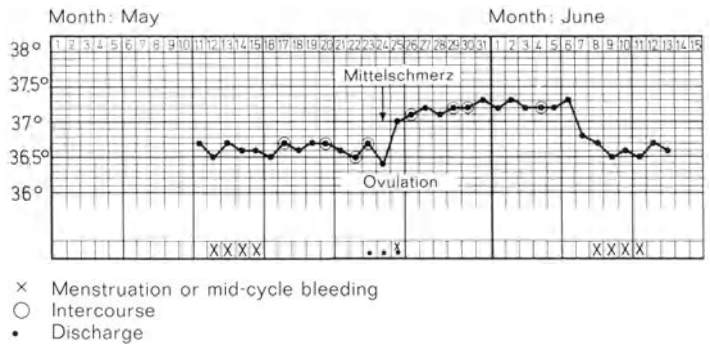


Fig. 69. A typical biphasic basal temperature curve

of menstruation. In 70–80% of women, the basal body temperature lies between 36.3 and 36.8° C postmenstrually, and premenstrually between 36.9 and 37.4° C (PLOTZ, 1950).

On the basis of information obtained from culdoscopic investigations (DOYLE, 1955) and laparotomies (RILEY *et al.*, 1955) permitting direct observation of ovulation, and from successful artifical insemination (WILLIAMS, 1964) it must be concluded that ovulation usually occurs 1–2 days before or after the temperature rise, but that it is also possible that ovulation may also take place earlier or even more than 2 days after the temperature rise (ABARBANEL, 1958; TOMPKINS, 1944, 1945). It is thought that follicular rupture often occurs at the same time as a fall in temperature, shortly before the temperature rises.

It is often difficult to fix the time of the temperature rise. In such cases, VOLLMANN (1940) suggests calculating the average temperature in the cycle and drawing a line through this temperature. The point at which this line intersects the rising arm of the curve is taken as the time of the temperature rise.

The temperature normally remains increased for 12–13 days (hyperthermal phase) and falls again shortly before menstruation to postmenstrual values.

The luteal phase is inadequate if the hyperthermal phase lasts less than 10 days. This leads to sterility. ISRAEL (1967) found a shortened luteal phase in 3.5% of a group of 942 women all of whom had sterile marriages. Probability of pregnancy is almost 97% when the hyperthermal phase lasts for 16 days or longer (BARTON, 1945). The basal temperature curve is monophasic when ovulation fails to occur. The frequency of anovulation is dependent on age and is highest during the first years after menarche (COLLETT, 1954; HARTMAN, 1962; DÖRING, 1963), during the climacteric period, and after gestation. It very rarely occurs in women between the ages of 30 and 35 (DÖRING, 1963). The basal temperature curve must be plotted for at least 3–4 months to determine when and if ovulation has occurred and if luteal insufficiency is possible. The patient uses the same table to record menstruation, intercourse, mittelschmerz, mid-cycle bleeding, increased intermenstrual discharge, dysmenorrhea and tension in the breasts. Possible causes of a rise in temperature, such as headache, indisposition, colds, etc. should also be recorded (Fig. 69).

a) Endometrium Biopsy

This is used to determine the secretory transformation and proliferation of the endometrium. The procedure can be performed in outpatient departments and without an anesthetic. Secretory transformation normally reaches a maximum on the 9th day after ovulation [i.e. 5 days before menstruation (HELD, 1945)], so that the biopsy should be taken around this time whenever possible. The danger of interrupting a pregnancy during this procedure is estimated to be very slight, but to avoid this risk, the 6th postovulatory day has been recommended for the biopsy (KISTNER, 1964). However, at this stage there are no typical luteal stromal changes (spiral arteries with periarterial, pseudodecidual stromal cells). The position of the uterus must be determined before the biopsy. Dilatation of the cervical canal is not necessary. The length of the cavity is also measured with the hysterometer before the biopsy. This measurement does not only reduce the danger of overlooking a perforation, but also gives an objective measurement of the size of the uterus. Uterine hypoplasia, which is much too often diagnosed especially in sterile patients, can be excluded when the cervical/cavity length is 7 cm or more. A flat narrow curette is used for the biopsy. NOVAK recommends the suction curette.

The biopsy is taken in the form of a smear from the posterior and anterior walls, the most suitable material being found in the fundal region. The mucosa obtained is fixed in absolute alcohol. In assessment of the endometrium, one must be aware that secretory transformation is no proof of ovulation having occurred, since a luteinized follicle can produce the same effect. The criteria for secretory transformation have already been discussed on p. 540.

Inadequate secretory transformation of the endometrium is found in 3–10% of sterile women (MARCUS, 1967). In a group of 102 sterile women, 6 (4.8%) showed no signs of a secretory transformation even five days before menstruation (HELD, 1948).

b) The Sims-Huhner Test (Post-Coital Test) (SIMS, 1869; HUHNER, 1913)

This is one of the most valuable means of investigating sterility. It is unfortunately not possible to compare the results of different authors, since the manner in which the test is interpreted varies greatly. A positive post-coital test makes collection of semen by masturbation unnecessary in the cases concerned, as this method of collection may distort the spermogramm due to psychological influences (DANEZIS, 1964). A positive test reveals that quantity and quality of sperms and cervical mucus are adequate, that the male partner is potent and the genital anatomical states of both partners allow for

deposition of sperms in the cervical region. In addition, it can have a therapeutic effect if performed at the right time. The optimal time is shortly before ovulation when the cervical secretion is at its optimum (SEGUY, 1933). The test can also be positive at other times, but no conclusions can be derived if it is negative at other points in the cycle (Fig. 70, ELERT, 1963). A definite prognosis cannot be made from a single negative result. The test must be repeated. Apart from the time at which the test should be performed, there is a series of other points to observe: intravaginal douching must not be performed for at least 48 hours before the test; 3 days' abstinence from intercourse is essential; the examination is performed a minimum of 6 hours and a maximum of 16 hours, ideally 8–12 hours, after intercourse. A good result before 6 hours have elapsed can give rise to false conclusions (DAVIDSON, 1961; STALLWORTHY, 1966). Sperms should not be fertile for more than 24 hours after intercourse. They retain their motility in cervical mucus for twice as long (HARTMAN, 1962). Nevertheless, weakly motile sperms have been observed in the cervical mucus even 134 hours after deposition (TYLER, 1961).

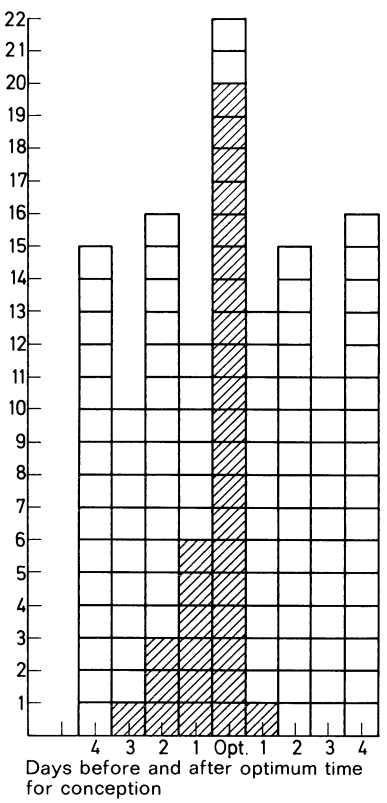


Fig. 70. Distribution of 106 Sims-Huhner tests around the optimum point for conception, determined with the aid of functional cervical diagnosis. Each square represents one Sims-Huhner test and each cross-hatched square represents a positive test. The restriction of positive tests to a few days is clearly apparent. (After ELERT, 1963)

The test is carried out by taking some mucus from the mucous collection in the region of the external os uteri and some from the lower cervical canal. This sample is put on a microscopic slide. The mucus is removed by means of aspiration with a suction pipette, or with the cervical mucus forceps after PALMER, or with a pincette (ROCK). The penetration test is considered positive if average magnification (400 ×) reveals at least 5 sperms moving progressively in one field. Not more than 4 leukocytes per field should be present if the cervical mucus is at its optimum, as it is at the time of ovulation (GRANT, 1958). A larger number of leukocytes at the time of ovulation indicates cervicitis. At any other time of the cycle, it is normal for leukocytes to be present in cervical mucus.

The appearance of the cervical mucus, however, provides no information about its biological value (glucose content, amino acids etc.), i.e. a clear cervical mucus can also be biologically inferior and lead to immobilization of the sperms.

A negative test must be repeated until the reason for it has been found. The result may be negative because the wrong time was chosen, because the cervical mucus was infected or biologically inferior, because there is a disturbance in potency or an anatomical defect impairing insemination, or because quality and/or quantity of the semen is inadequate. If pathogenic organisms are present, bacteriological examination of the cervical mucus and resistence tests are indicated. It is still open to discussion whether an antigen-antibody reaction due to AB0 incompatibility can occur between sperm and cervical mucus (BEHRMAN, 1960, 1967; WHITELAW, 1962). Antibodies which react cytotoxically to sperms of the husband have been demonstrated by immunofluoroscopy in the cervical mucus and blood of sterile women (PARISH, 1968). So-called lethal factors of unknown nature have been reported in the vagina and cervix (MASTERS, 1961).

The fern test (PAPANICOLAOU, 1946; RYDBERG, 1948; CAMPOS DA PAZ, 1953) should be performed on cervical mucus at the same time as the Sims-Huhner test (p. 627, Fig. 71).

At the time of ovulation, a large quantity of thin mucus of high elasticity and reduced viscosity is produced under the maximal action of estrogens. The viscosity is examined by the so-called spinnbarkeit, which is greatest at the time of ovulation (p. 627, Fig. 72).

Fig. 71. Dried cervical mucus taken from a sexually mature woman at the time of ovulation (fern-leaf phenomenon)

c) Pertubation, Hysterosalpingography and Laparoscopy/Culdoscopy

These different examination techniques are complementary rather than competitive. The Rubin test, which reveals whether the tubes are patents, is applied first (RUBIN, 1920, 1932, 1947; SEMM, 1969). It is one of the most important and practical methods in the investigation of sterility. When carried out properly it can reveal whether both tubes are patent or just one, whether a stenosis is present and whether tubal peristalsis is adequate (HELD, 1940). The test tells us nothing, however, about the minute physiology and anatomy of the tubes, e.g. transport of the ovum, the relative positions of fimbrial funnel and ovary, which are important to fertility, localization of a possible obstruction. The apparatus we prefer is the model of SEMM (1969). It permits the necessary simultaneous volumetric and manometric control. The pertubation apparatus developed by Fi-

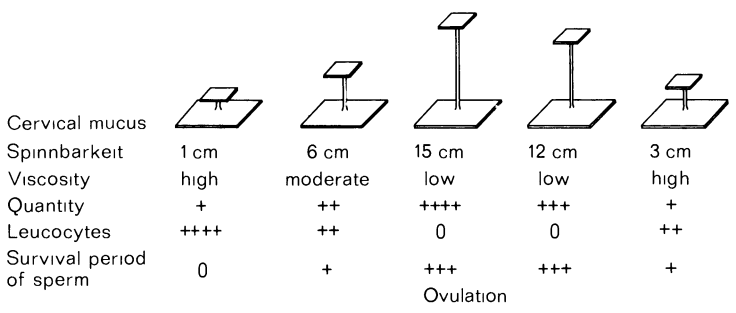


Fig. 72. Changes in the cervical mucus during the cycle. (After COHEN, 1952)

KENTSCHER and SEMM (SEMM, 1969) and by SHARMAN are suitable for scientific and clinical use. A vacuum adapter is used for obturation.

If full use is to be made of the diagnostic possibilities offered by these methods, and false results avoided, the following points must be observed. The best time is between the 4th and 7th days after menstruation. Every pertubation must be preceded by a gynecological examination to obtain information about the state of the portio, position of uterus and state of the ovaries (see contraindications). To exclude any spastic processes in the region of the uterus and tubes, a general anesthetic is given or the patient receives 1 ampoule Spasmalgin 30 min before the examination and 0.5 mg nitroglycerine on a lump of sugar just before the actual pertubation. The portio should be pulled down as little as possible to avoid altering the relative positions of uterus and tubes. If a pressure of 100 mg Hg or 150 mm Hg is reached with a flow velocity of 30 ml/min, the flow of CO_2 must be stopped for 1 min to avoid tubal spasm due to too rapid an increase in pressure. The pressure should not exceed 200 mm Hg. With a flow velocity of 30 ml/min, normal tubes are patent at a pressure between 40 and 90 mm Hg. Stenotic processes are present if persufflation first occurs at a pressure of 140–200 mm. Tubes of normal diameter allow CO_2 flow at a rate of 180 ml/min without causing pressure to rise over 120 mm Hg. This rate of flow thus allows the diameter of the tubes to be examined at the end of the investigation. Since 250 ml CO_2/min are expired through the lungs, 250 ml CO_2/min can be insufflated with no danger to the patient. Not more than 200 ml CO_2 are required for examination of tubal patency. If at least 90–120 ml CO_2 reaches the free abdominal cavity, the subdiaphragmatic collection of gas characteristically gives rise to shoulder pains, usually on the right side. Monitoring by X-ray screening is essential and allows the phenomenon to be objectified. The lower abdomen is auscultated as persufflation is performed. This will not only detect the localization of an obstruction, but the character of the sound of the flow will also make it possible to localize any stenosis. If there is no patency at a pressure of 200 mm Hg this indicates bilateral tubal occlusion. A bimanual gynecological examination should follow every pertubation so that any pneumosalpinx present can be diagnosed. The liver dullness should be percussed before and after the procedure, and X-ray screening performed within 1–2 hours.

Pertubation is contraindicated in the presence of pelvic inflammation and during genital bleeding (air embolism, spread of endometrium).

Though the reason is not understood, the Rubin test has a therapeutic effect in addition to its diagnostic significance. ISRAEL (1967) observed that 4% of his patients with an average sterility duration of 3.5 years became pregnant after pertubation. Other authors report a success rate of 20–30%, which is improbably high (RUBIN, 1920; SHARMAN, 1954; SCHILDBACH, 1950).

A single negative test does not prove a definite occlusion. Although RUBIN (1947) is not of the same opinion, three negative results can still be incorrect. JEFFCOATE (1953) reported 25 pregnancies after obtaining 3 negative pertubations in each case. This is also applicable to the hysterosalpingography (PEEL, 1964; STALLWORTHY, 1966). Examination of the patency at the junction of uterus and tube is very difficult, since the lumen at this point has a diameter of only 100 µm (ROCKER, 1964), and in 75% of cases the course of the interstitial part of the tube is angular (SWEENEY, 1962). In addition, further narrowing occurs under the influence of estrogens, particularly at the time of the examination (HARTMAN, 1962). When the results of pertubation are interpreted, it must be borne in mind that a negative result can be due to a functional closure as well as to an anatomic anomaly. Functional closure is, however, often a result of organic changes. A positive result does not necessarily indicate normal conditions, since peritubal adhesions or endometriosis may still be present.

A *hysterosalpingography* (SEMM, 1969) must be performed to localize the site of obstruction when the tubes are occluded. We do not think that should be carried out routinely in every case of sterility. Unfortunately, the ideal contrast medium has not yet been discovered. Water-soluble media are too rapidly absorbed, so that it its impossible to obtain an X-ray picture after 24 hours, which is essential for the detection of peritubal adhesions. On the other hand, granulomatous formations are more frequent with oil-soluble contrast media.

A *culdoscopy* or *laparoscopy* (DOYLE, 1951; FRANGENHEIM, 1959) is indicated when the patient fails to become pregnant within one year even when the tubes have been shown to be patent and no other sterility factors can be found. It is also indicated when pertubation and hysterosalpingography give obscure results or show that the tubes are not patent.

It allows detection of endometrial or inflammatory changes in the pelvic cavity which cannot be determined by pertubation and hysterosalpingography. This is especially true of peritubal adhesions, which impair tubal motility and the mechanism of collecting the

ovum. Thus, in a group of 82 sterile women with normal clinical and hysterosalpingographic findings, culdoscopy revealed pelvic and/or peritoneal changes in 35% and endometriotic changes in 29% (RIVA, 1959). Only 20–25% of sterile women patients have normal culdoscopic findings. Postinflammatory changes are found in 48% and 26–40% prove to be affected by endometriosis in the small pelvis (KISTNER, 1962). "Idiopathic sterility" should therefore only be diagnosed after culdoscopic/laparoscopic examination. If pertubation and hysterosalpingography show that the tubes are occluded the patency should be tested during laparoscopy by the injection of a colored solution (hydropertubation) of, for example, methylene blue or indigo carmine, into the uterus (so-called three-fold examination of tubal patency). Culdoscopy revealed patent tubes in 30% of patients in a group of women with tubes shown to be occluded by means of pertubation and hysterosalpingography (PERETZ, 1961). The diagnosis of occluded tubes should therefore only strictly be made when the tubes are shown to be obstructed by hydropertubation during culdoscopy or laparoscopy.

4. Treatment of Female Sterility

Improvement of the quality and quantity of cervical mucus is achieved by administering an estriol preparation such as Ovestin in a dose of two tablets of 1 mg daily from days 4–14 of the cycle. Estriol has the advantage that it acts practically exclusively on the cervical mucosa (PUCK, 1958). Due to the strong central inhibitory action on the hypothalamo-hypophyseal system, ethinyl-estradiol preparations (Lynoral, Progynon C) and diethyl stilbestrol should only be given in very small doses: e.g. 0.02 mg ethinyl estradiol, or 0.25 mg stilbestrol daily on days 6–12 of the cycle. Higher doses, such as 0.04 ethinyl estradiol taken daily from day 2 to day 8 of the cycle cause ovulation to be postponed by a week. This form of treatment is thus suitable for displacing ovulation where ovulation occurs during the menstruation phase in the presence of biphasic polymenorrhea.

Uterine Hypoplasia, which is an uncommon cause of sterility, can be influenced pharmacologically by producing a pseudocyesis. Treatment with estrogen-progesterone for 12 weeks can cause the sound length to increase by about 2 cm. For example, Anovlar is given from the 22nd day of the cycle; 1 tablet is taken daily, and the dose is increased by one tablet every 10–14 days until it reaches 3 tablets daily (for at least 3 month).

Surgical correction of *malformations* of the uterus by the Strassmann plastic operation is justified only in cases of habitual abortion.

Enucleation of a fibroid is only indicated in the absence of other sterility factors in the subject and the husband. The success obtained in carefully selected cases is claimed to be surprisingly good (BROWN, 1956; DAVIDS, 1959). STEVENSON (1964) observed full-term pregnancies in 50% of cases.

TANNER (1953) recommends conservative treatment of genital tuberculosis with the tuberculostatic medicaments of choice: streptomycin, PAS, ethambutol, rifampicin, isoniazid.

Hypoplasia of the Endometrium is most probably connected with tubal dysfunction. In such cases where no other sterility factors are present a three-month cyclic ovulostatic therapy is often successful, acting perhaps via a hypophyseal rebound mechanism (KISTNER, 1964).

Inadequate Secretory Transformation of the endometrium can be corrected by either substitution or stimulation treatment (Table 60). We prefer to use an acetoxy-progesterone derivative in oral substitution therapy, because of the slight androgenic action of nortestoids on the fetus. Stimulation with a total of 5000–15000 IU HCG will cause a corpus luteum of menstruation to be converted into one typical of pregnancy (BROWN, 1947; JAYLE, 1965).

Table 60. Treatment of inadequate secretory transformation of the endometrium

A. Substitution treatment
a) 200–250 mg progesterone + 10 mg estradiol 2–3 days after rise in temperature
e.g. Sistocyclin (200 mg progesterone crist. + 10 mg estradiol monobenzoate)
Primosiston (250 mg hydroxy-progesterone capronate + 10 mg estradiol benzoate)
Withdrawal bleeding 12 days later if pregnancy has not occurred
b) Planovin or another inhibitor of ovulation (4 mg megestrol acetate + 50 µg ethinyl estradiol), 1 tablet daily on days 18–25 of cycle
B. Stimulant treatment
Chorionic gonadotropin (HCG)
e.g. 4 doses of 1500 IU HCG, administered on days 4, 6, 8, and 10 of the hyperthermic phase, or
3 doses of 5000 IU HCG, administered on days 3, 5, and 7 after rise in temperature
Clomifene citrate
50 mg/day on days 5–25 of cycle

Until a few years ago, *anovulation* was a therapeutically unconquerable obstacle in practically all cases of sterility. This state of affairs

has changed fundamentally since human pituitary gonadotropins (p. 600) and clomifene citrate have become available (p. 599).

Treatment of Anovulatory Sterility with Gonadotropins is delicate and expensive also (p. 599ff.). Patients for this treatment must be carefully selected, the choice based on general examination and gynecological and endocrine investigations. Absence of other sterility factors is essential. Estimation of the hypophyseal gonadotropins (HPG) is essential. Only women with inadequate or normal amounts of gonadotropins can be considered. Treatment of hypergonadotropic anovulation with gonadotropins is pointless. On the other hand it is of no importance whether a primary estrogen deficit is present or not. Pretherapeutic estimation of urinary estrogens is therefore not necessary. The action of estrogens is monitored before and during treatment by means of Shorr smears, fern test, spinnbarkeit of cervical mucus and diameter of the external os uteri. An endometrium biopsy and a progesterone test are part of the investigation.

The response of the ovaries to gonadotropic stimulation varies widely from one patient to another. The dosage must therefore be adjusted to the individual. Special caution is required in patients with polycystic ovaries, since such ovaries are particularly sensitive to gonadotropic stimulation (p. 615). We do not use the gonadotropin sensitivity test (CROOKE, 1965). We begin with a low dose because of the danger of overstimulation, following the schedule given in Fig. 59.

The patient receives 75 IU HMG/day, e.g. 1 ampoule Pergonal. As soon as the daily Shorr smears, the cervical mucus, and the diameter of the os uteri show a good reaction to estrogens, the patient is given 5000 IU HCG daily for 3 days (e.g. Pregnyl i.m.). This level is usually reached after 7–10 days. Before the dose of HCG is increased, it is essential to perform a careful gynecological examination to make sure that the ovaries are not painful or enlarged. If this should occur, i.e. if overstimulation has occurred, only 1000 IU are given daily for 2–3 days. This reduces the danger of rupture of an induced ovarian cyst. Intercourse should take place a few hours before and 12 hours after administration of HCG.

The HMG doses are doubled in cases in which the doses mentioned have caused inadequate stimulation of the ovaries. Thus the patient receives 150 IU i.m. daily for 10–15 days. Exceptionally this dose is increased to 225 IU HMG (2–3 ampoules Pergonal). As soon as there is evidence that vagina and cervix are reacting well to estrogens and there is a definite rise in urinary estrogens, 5000 IU HCG are given daily for 3–5 days.

From the results available, it can be assumed that human follicular stimulating hormone acts according to the all-or-nothing law. However, there is only a very small difference between the effective dose and that which leads to overstimulation.

The success, measured by release of ovulation, is very good in correctly chosen patients. Ovulation can be produced in all cases. VAN DE WIELE (1965) observed that 60% of these sterile patients became pregnant. More than half of them, however, aborted, and of the viable children there were as many multiple pregnancies as single pregnancies. HMG/HCG treatment has resulted in pregnancy even in patients with Sheehan syndrome who had been amenorrheic for years.

The danger of overstimulation with the formation of cysts and rupture must not be underestimated even when patients are correctly chosen and doses are cautiously regulated. Multiple ovulations are frequently induced, which is a further disadvantage as they can lead to multiple pregnancies, which in turn result in increased abortion and prematurity rates, an increased incidence of toxemia, and more cases of pathologic presentation and abnormal births with corresponding danger to mother and child. It is therefore absolutely essential to make the couple aware of the increased risks before treatment is started. A sustained action is not achieved with this form of therapy. Another ovulation can only be produced by renewed stimulation.

Clomifene citrate given to release ovulation has a number of advantages over *menopausal gonadotropins*: it can be given orally, the number of multiple pregnancies is smaller, there is a lower risk of overstimulating the ovaries, and a better chance of ovulation occurring spontaneously after withdrawal of the treatment. However, there are fewer indications (GREENBLATT, 1961; KISTNER, 1965). On the basis of experience obtained with a few thousand women, the indications can be confined within certain limits.

Clomid (clomifene citrate, chloramiphene, MRL-41) is closely related chemically to the synthetic estrogens, stilbestrol and chlortrianisen (Tace). It is also chemically similar to MER-25, which is an effective antiestrogen but is toxic. Clomid is given orally in the form of a citrate salt. Its spectrum of activity varies in the individual species (SCHREINER, 1967).

In the ovariectomized rodent it has a weak estrogenic action, but inhibits the action of

estrogens when the two drugs are given together. In higher doses, it also markedly reduces the excretion of gonadotropins in the urine. Thus, clomid has both estrogenic and anti-estrogenic effects at the same time in the rodent, and has an inhibitory action on ovulation. It has an entirely estrogenic action in Rhesus monkeys.

In contrast to its effects in rodents, clomid's main effect is to promote ovulation in the human. In the woman, its action is primarily anti-estrogenic, so that it eliminates the effect of an ovulation inhibitor, for example. Gonadotropin excretion is increased and the corpus luteal phase is prolonged in eucyclic women when clomid is taken from days 5–25 of the cycle.

In contrast to MER-25, no toxic effects have yet been observed in women when therapeutic doses are used.

Clomid has proved to be a very effective drug in promoting ovulation in carefully chosen anovulatory patients. In these women, the endogenous production of estrogens is usually adequate and gonadotropin excretion is normal or at the most slightly reduced but not cyclic. Pituitary and ovaries must be intact anatomically, and adrenocortical and thyroid function normal. Due to the absence of a cyclic production of gonadotropins, these women show a cessation in follicular maturation, the endometrium is well developed, and they may show the picture of glandular cystic hyperplasia. The progesterone test is positive.

These patients present clinically with oligomenorrhea or amenorrhea, often associated with sterility due to oligo- or anovulation. This large group of forms of oligo-amenorrhea due predominantly to hypothalamic factors includes: secondary amenorrhea with psychological causes, especially of the normogonadotropic type, undefinable normogonadotropic secondary amenorrhea such as the so-called Stein-Leventhal syndrome, oligomenorrhea with the clinical picture of metropathia haemorrhagica often associated with mastodynia. Furthermore, this group also includes sterility due to hypothalamic oligo- or anovulation found in couples with otherwise normal fertility factors.

Treatment of hypogonadotropic amenorrhea with clomid is not very promising, but the attempt is justified in cases with an otherwise normal endocrinological state. This is applicable, for example, in the cases of secondary galactorrhea such as is found in the Chiari-Frommel syndrome. Panhypopituitarism, which may occur in an advanced Sheehan syndrome or with intrasellar tumors, does not respond to treatment with clomid.

Examination of the retina and sella turcica must therefore also be included in the pretherapy examination. Treatment with clomid is also unsuccessful in hypergonadotropic amenorrhea, for example in premature menopause or gonadal dysgenesia.

The dose is dependent on the patient and on the sensitivity of the ovaries to gonadotropins. An initial treatment with 50 mg clomid is advisable for 4 to 5 days starting between the 5th and 9th days after onset of spontaneous menstruation or bleeding induced by a gestagen (Fig. 73). Ovulation occurs between the 2nd and 41st days after onset of treatment in about 70% of women treated with clomid (JOHNSON, 1966) but usually after 8 to 12 days. Cyclic treatment can be repeated after an interval of at least 4 weeks, and before a new treatment is started, a pregnancy or corpus luteal cyst must be excluded. The highest success rate is obtained in women with the Stein-Leventhal syndrome (approximately 78–82%). A low dose of 50 mg daily is chosen, due to the sensitivity of the ovaries in these women. Pregnancy occurs in over 50% of the patients in whom sterility is due to hypothalamic oligo- or anovulation and in whom other fertility factors are normal. However, about 7.5% of the pregnancies arising as a result of clomid therapy are multiple pregnancies (JOHNSON, 1967). The abortion rate in these women is about 22%, i.e. much higher than in the average population, but similar to that secondarily sterile women

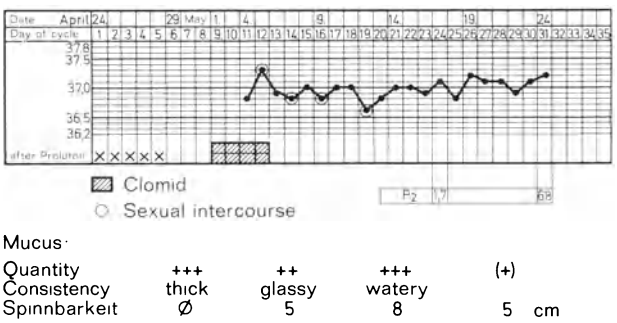


Fig. 73. Temperature curve of a patient treated with clomid (after WYSS, 1967). Next check-up on 27th June = day 65 of cycle, Gravindex positive, clinical examination revealed a 2-month pregnancy, subjective indications of pregnancy

(BUXTON, 1958). Among 672 pregnancies, including those ending in abortion, there were 1.8% newborns with malformations. This figure is not higher than that in a group of untreated fertile women. A low dose is important in the treatment of sterility. A higher dose of clomid can suppress ovulation in the human as it does in rodents, whereas even larger amounts of clomid can cause the cervical mucus to become thicker, viscous and scanty.

Treatment with 100 mg daily for 4–5 days can result in ovulation in individual cases in whom no success has been achieved with low doses.

The production of ovarian cysts, chiefly luteal cysts, is the greatest potential danger of treatment with clomid. Formation of ascites or rupture with intraperitoneal hemorrhage may then occur; such cases have been described. Although there is only a slight danger of this (about 5%) when treatment is of short duration, the risk increases when the duration is more than 10 days and treatment is repeated. The duration of therapy must therefore not exceed 5 days per cycle, and daily doses of over 100 mg must not be given for longer than 5 days. Palpation of the pelvis before, during and after the administration of clomid is therefore absolutely essential. Ovarian cysts that are induced usually disappear spontaneously 3 to 4 weeks after the withdrawal of clomid.

About 15% of the women suffer from hot flushes as a side effect of treatment with clomid. Other side effects which arise considerably less commonly (5%) are: nervousness, headache, giddiness, visual disturbances, loss of hair, nausea, sleeplessness, weight increase, abdominal complaints and pollakisuria.

It is definitely essential to select patients critically by means of pretherapeutic investigations and to monitor the patients during treatment. Apart from the low success rate when patients are not chosen carefully, noncritical use of clomid can also result in serious complications.

The mechanism of action of clomid is still open to discussion. It is certain that in suitable conditions, clomid causes a significant increase in LH activity, whereas FSH excretion is only slightly changed (KELLER, 1966; Fig. 74). Stimulation of LH appears to be the predominant mode of action of clomid. The fall in the FSH/LH quotient (Fig. 75) leads to ovulation. An increased estrogen excretion has been described before the peak of LH excretion, so that conditions analogous to spontaneous ovulation seem to be present. Individual results indicate that clomid acts as a competitive inhibitor to estrogens by occupying specific cell receptors for estrogens. This effect would explain the observation that clomid therapy fails to produce estrogenic effects on the uterus, vagina and breasts, despite normal estrogen levels (antiestrogenic effect). Different receptor organs have been considered for clomid (ROY, 1963, 1964). Certain experiments *in vitro* show a direct influence of clomifene on ovarian enzymes taking part in the biosynthesis of estrogens; it accelerates aromatization of androstenedione and testosterone and the formation of estrone and estradiol by inhibiting cytochrome reduction with an accumulation of NADPH (HAGERMAN, 1966). There does, however, appear to be a direct hypothalamic action. This was demonstrated in investigations in a 30-year-old woman with primary ovarian amenorrhea, showing that the LH-stimulating action of clomid is connected with a definite estrogen level but not with the ovary itself (Fig. 75) (KELLER, 1966).

A trial with thyroid drugs is justified in sterile patients with clinical hypothyroidism or with laboratory findings at the lower normal limits.

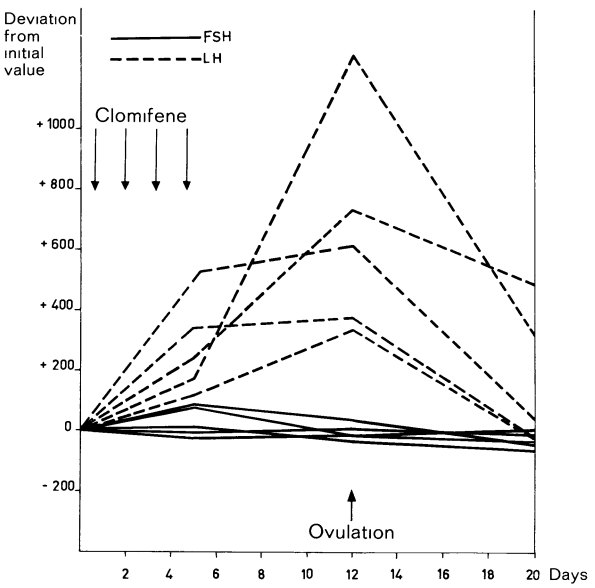


Fig. 74. Percentage increase and decrease in the excretion of FSH and LH with the urine in secondary amenorrhea following clomifene medication (KELLER, 1966)

Treatment with prednisone in a dose of 10–15 mg daily may result in ovulation in borderline adrenal hyperplasia (p. 612). This hyperplasia can present clinically as anovulation, less commonly as a reduced luteal phase, as secondary amenorrhea, hirsutism, acne vulgaris and even as mild hypertrophy of the clitoris.

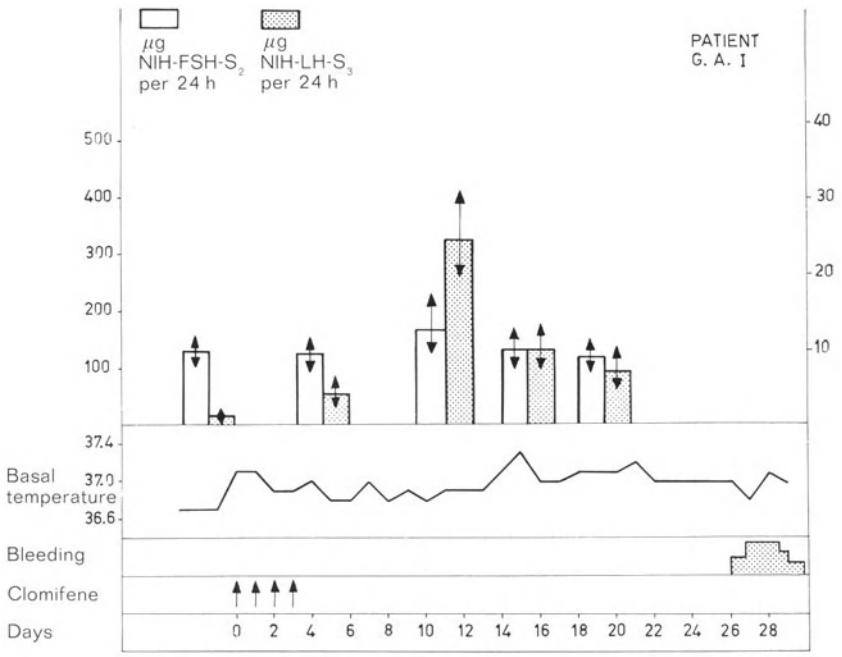


Fig. 75. Excretion of FSH and LH in secondary amenorrhea and anovulation before and after clomifene medication (KELLER, 1966)

Prompt treatment of salpingitis with antibiotics is of decisive importance for future reproductivity. We were not convinced by the material obtained from the Gynecological Clinic in Zurich of the advantages of additional cortisone treatment, which is theoretically justifiable (FUMASOLI, 1969). Residual states, frequency of recurrences, and fertility were no less than were obtained with antibiotic treatment and subsequent resolutive physiotherapy.

The prognosis of fertility after salpingitis is dependent on the state of the tubes at the onset of treatment. This is especially applicable to tuberculous salpingitis (p. 622). Palpable ovarian tumors exclude a later pregnancy. Treatment of genital tuberculosis is carried out with tuberculostatics of first choice.

If all surgical experience is considered, the results of *plastic operations on the tubes* seem to be consistent. These operations are: salpingolysis, fimbriolysis, salpingostomy, end-to-end anastomosis and tubal implantation. Pregnancy later arose in 11 to 17% of patients subjected to these operations (PALMER, 1952; GREENHILL, 1956; SIEGLER, 1956; KISTNER, 1964; BICKENBACH, 1967). Better results reported by individual authors are probably based on a special selection of patients, different tubal pathology and different surgical techniques (MARCUS, 1967; MROUEH, 1967). Tubal occlusion after generalized salpingitis has a considerably worse prognosis than after surgical sterilization.

A pelvic *endometriosis* must always be suspected when the cause of sterility cannot be found. The tubes are usually patent. If the laparoscopy is positive and no other incurable sterility factors can be detected, the diagnosis must be made histologically before treatment is started.

Two kinds of treatment are available, medical and surgical.

Medical Treatment of Endometriosis

The therapeutic procedure introduced by Kistner in 1956 is based theoretically on the observation that pregnancy can retard the development of endometriosis, probably through causing necrosis of the endometrium converted into decidua. The curative effect of pregnancy on endometriosis has, however, become controversial in the light of recent investigations (MCARTHUR, 1965). Treatment consists of induction of a pseudocyesis lasting at least 6–9 months (possibly 12–24 months) by giving progestagens continuously in increasing doses (Table 61).

The side effects are due mainly to the estrogenic components. They are similar to the effects observed in cyclic ovulostatic treatment, but are more frequent and severe. Nausea can be alleviated by taking the tablets with milk, or by using an antacid or antiemetic. Water retention is treated by a saltfree diet, possibly combined with a diuretic. Treatment with

progestagens alone must be substituted if side effects are severe, such as very painful breasts, bad headaches, growth of uterine fibroids, severe unrest, irritability and insomnia. Treatment with progestagens often leads to breakthrough hemorrhage and results in aggravation of skin changes in women with a tendency to acne and with a seborrheic skin. There is an increased risk of thromboembolism.

Table 61. Medicamentous treatment of endometriosis

Oral inhibitors of ovulation or progestagen e.g. Primolut-N Beginning on day 22 of cycle 1 tablet daily for 2 weeks 2 tablets daily for 2 weeks 3 tablets daily for 2 weeks 4–5 tablets as long-term therapy
Primosiston 1.0 ml (250 mg hydroxy-progesterone capronate and 10 mg estradiol benzoate) i.m. once weekly. Increase dose by 0.5 ml every six weeks or if breakthrough bleeding occurs
Depo-Provera 2.0 ml (100 mg medroxy-progesterone acetate) 4 ml i.m. every two weeks; then 4.0 ml every 4 weeks. Oral estrogens should be added if breakthrough bleeding occurs

It is essential to start hormonal treatment without delay in a case where endometriosis has been diagnosed and pregnancy has not occurred within a short time. About 85% of women treated by this regime show a temporary improvement – symptoms disappear and tumors regress (GRANT, 1964). The average duration of remission, however, is only 8–9 months (RIVA, 1962), although remissions lasting for 52 months have been observed (KISTNER, 1964). Some 15–20% show a relapse even 6 months after discontinuation of treatment (RIVA, 1961, 1962; GRANT, 1963, 1964). Information on the frequency of conception after discontinuation of treatment varies between 10–47% (RIVA, 1962; KISTNER, 1966). Conception most often occurs with the second or third spontaneous ovulation.

Results obtained with conservative surgical treatment are somewhat better. On average 52% of 844 women in a pooled group became pregnant postoperatively. This value varied between 39–70% (MARCUS, 1967).

In our opinion, the conservative surgical approach is preferable to medical treatment when these results are compared and the long duration, high costs, frequent side effects and rapid and frequent remissions associated with medical treatment are considered. Conservative surgical treatment means careful excision of all endometrial foci if this is anatomically possible. Foci which cannot be excised because of their anatomical position, e.g. on the rectum or bladder, must be sloughed off electrically. Peritubal and periovarian adhesions must be detached, a fixed uterus must be anteflexed, and finally the presacral nerve must be resected. Postoperatively, the patient receives uninterrupted ovulostatic treatment for 3–4 months, according to the schedule for conservative medical treatment, to prevent growth of any endometrial foci which may have been left behind (MCCOY, 1963; GREEN, 1966).

I. Endocrine-Active Ovarian Tumors

Blastomatous proliferation of different ovarian tissues can lead to the formation of endocrine-active tumors, which can produce steroids, chorionic gonadotropin or thyroxine in quantities giving rise to characteristic clinical pictures (Table 62). Various changes arise according to the predominant hormone secreted: early isosexual differentiation or disturbances, heterosexual changes in the form of defeminization and virilism, Cushing's syndrome, gestotic changes, or hyperthyroidism. Removal of the tumor causes partial or complete regression of these changes.

It is often very difficult to classify these cases histologically or histogenetically if the tumor tissue is very poorly differentiated. In some conditions classification is possible with some degree of probability only when the clinical picture is also taken into consideration. Apart from this, the histological picture of the same tumor may vary widely in different sections.

On the other hand, the endocrine activity of a tumor is no absolute criterion for classification, since different types of tumor cells can demonstrate a similar hormone secretion, i.e. morphological changes need not be associated with changes in the enzyme pattern.

For practical reasons, endocrine-active ovarian tumors are classified by clinical effect in the following discussion (Table 63).

1. Predominantly Estrogen-Producing Tumors ("Feminizing" Tumors)

The estrogen-producing *granulosa-theca cell tumors* are the most common hormone-active ovarian tumors. This term was derived by VON WERDT (1914). They were first recognized as true tumors by LOEFFLER and PRIESEL, however, in 1932, and they described them as

Table 62. Endocrine syndrome and ovarian pathology. (Modified from MORRIS and SCULLY, 1958)

Hormonal status	Syndrome	Pathologic clinical findings	Ovarian pathology
Estrogen excess (or only estrogen)	Feminization estrinism	Anovulation, oligo-amenorrhea, meno-metrorrhagia	
	1. Pseudopubertas praecox	1. Menarche and development of secondary sexual characteristics before 8th year of life, anovulatory cycles accelerated skeletal growth	Follicular cysts (follicular persistence)
	2. Dysfunctional bleeding	2. Breakthrough bleeding in anovulatory cycles; hyperplasia or glandular/cystic hyperplasia of the endometrium; hypertrophy of the myometrium	Polycystic ovaries Granulosa tumors [in exceptional cases lipoid-cell tumors (folliculome lipidique Lecène)]
	3. Postmenopausal bleeding	3. Hyperplasia or glandular/cystic hyperplasia of the endometrium, cornification of vaginal epithelium	Brenner tumors (in exceptional cases cystadenomas, ovarian carcinoma
Androgen excess	a) Defeminization	a) Anovulation, oligo-amenorrhea, meno-metrorrhagia; atrophy of breasts and regression of secondary sexual characteristics, atrophy of endometrium, myometrium and vaginal epithelium, acne vulgaris	Polycystic ovaries with hyperthecosis Arrhenoblastomas Gynandroblastomas Hilus-cell tumors Masculinovoblastomas (virilizing lipoid-cell tumors)
	b) Virilization	b) Amenorrhea, hirsutism, hypertrophy of clitoris, receding hairline, prominentia laryngea and deepening of voice, masculine habitus	Gonadoblastomas (in exceptional cases pseudomucincystomas, Brenner tumors, primary and secondary ovarian carcinomas
Corticosteroid excess	Cushing's syndrome with or without virilization	Obesity of trunk, striae, polycythemiae, diabetic metabolism, osteoporosis	Lipoid-cell tumors
Thyroxine excess	Hyperthyreosis	Anovulation, oligo-amenorrhea, nervousness, tachycardia, exophthalmos, hyperhidrosis, muscular weakness, increased basal metabolism	Struma ovarii (teratomas)

Table 63. Endocrine-active ovarian tumors

1. Tumors producing mainly estrogens (feminizing tumors)
   Granulosa tumors (feminizing mesenchyma after NOVAK), theca-cell tumors = fibroma thecacellulare xanthomatoides ovarii Löffler-Priesel
   Folliculoma lipidique (Lecène = luteinized granulosa tumor)
2. Tumors producing mainly androgens (virilizing tumors)
   a) Androblastomas
      Arrhenoblastomas (Sertoli-Leydig cell tumors)
      Hilus-cell tumors (Leydig-cell tumors)
      Virilizing lipoid-cell tumors (masculinovoblastomas, adrenal-remnant tumors, hypernephroid tumors)
      Gonadoblastomas
   b) Mixed tumors
      Gynandroblastomas
3. Tumors producing corticosteroids
4. Tumors producing chorionic gonadotropins
   Chorioepitheliomas
5. Tumors producing thyroxine
   Struma ovarii (teratomas)

fibroma thecacellulare xanthomatoides ovarii. They are also known as *feminizing mesenchymomas* (NOVAK, 1952) on the basis of their clinical effect and presumptive origin of ovarian mesenchyme. Luteinizing granulosa-theca cell tumors are termed *luteomas*. The frequency of granulosa-theca cell tumors varies between 3 and 7.5% of all ovarian tumors according to the statistics considered (MORRIS and SCULLY, 1958; HERTIG and GORE, 1961; SANTESSON, 1947). Information about the percentages of granulosa cell tumors, theca cell tumors (thecomas) and mixed tumors must be evaluated with reservation (HERTIG and GORE, 1961) since there are no serial section examinations for the majority of these tumors. Granulosa-theca cell tumors are probably the most common (MORRIS and SCULLY, 1958). They are seldom observed before puberty (5%), and are encountered with roughly the same frequency during sexual maturity (55%) and in the postmenopause

(40%) (MORRIS and SCULLY, 1958). They vary in size between a few millimeters and the size of a man's head and are unilateral in 95% of cases (Fig. 76a).

The cut surface of granulosa-cell tumors (Fig. 76b) is homogeneous and soft. There may be necrotic hemorrhagic or cystic areas. Call-Exner bodies are typical histological findings.

Theca-cell tumors (Fig. 77) are solid and firm. The cut surface is yellowish. Characteristic microscopic findings are hyaline, fibrous patches arranged between twirled spindle cells rich in cytoplasm.

In addition, there are undifferentiated sarcoma-like forms which can only be classified on the basis of differentiated sections. It has been shown that granulosa-theca cell tumors have a high ability to convert various precursors of estrogen synthesis into estrogens (MARSH, 1962). Results of the chemical measurements of estrogens in tumor tissue performed by various authors vary vastly. KECSÉS (1963), using paper chromatography, found unusually high values, presented in Table 64. Other authors found considerably lower estrogen concentrations (SAAMELI and JKLÉ, 1959; BRUK, 1960).

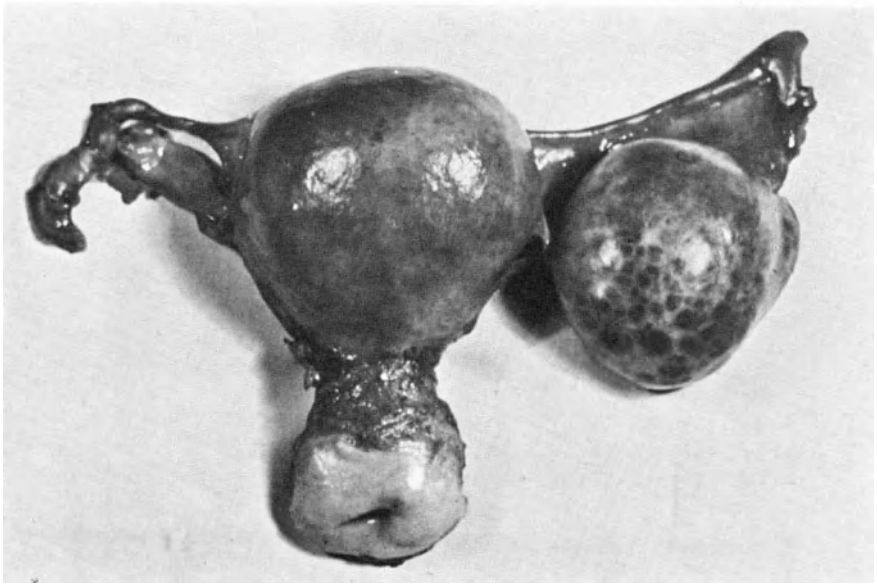


Fig. 76a

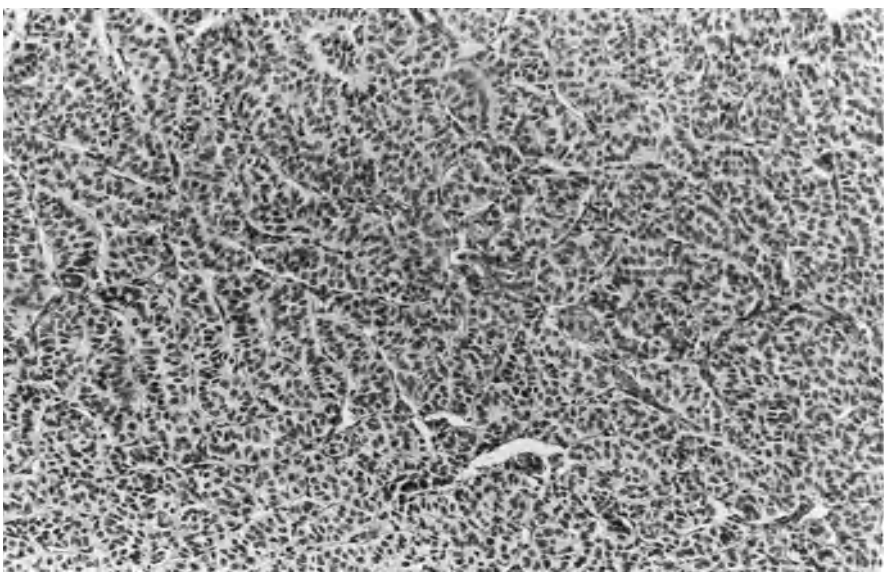


Fig. 76b

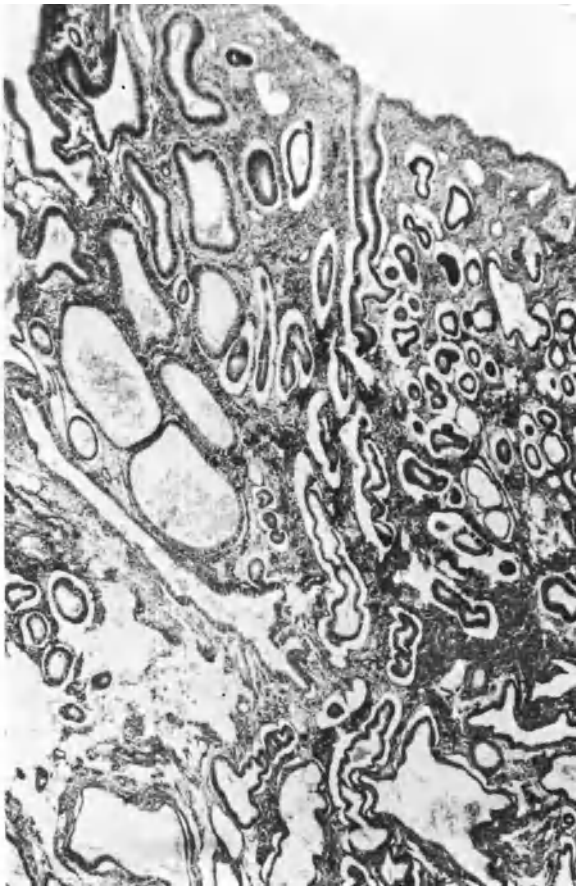


Fig. 76a–c. Left sided granulosa-cell tumor (a) with pseudotubular structure (b). Recurrent uterine bleeding 18 years after the menopause. Endometrium: glandular-cystic hyperplasia (c)

The figures quoted for frequency of local recurrences and metastases vary between 10–33%. Recurrences characteristically arise late (VARANGOT, 1937; DIDDLE, 1952), 15–25 years after removal of the primary tumor (MORRIS and SCULLY, 1958). This is also supported by KOTTMEIER's statistics (1952), which show that 17% of recurrences arise within the first 5 years, and 41% after 5 years. The follicular form is thought to have a considerably better prognosis than sarcomatoid types (KOTTMEIER, 1952), with 22% recurrences as against 71%. These tumors are extremely radiosensitive. There are different ways of looking at the question of whether there is any connection between the granulosa-theca cell tumor and carcinoma of the body of the uterus. Different investigators (INGRAM and NOVAK, 1951; DOCKERTY and MUSSEY, 1951; MANSELL and HERTIG, 1955) have found carcinoma of the endometrium in 14–15% of women with granulosa-theca cell tumors, and in as many as 24 or 27% of cases where the women were over 50. On the other hand, SJÖSTEDT and WAHLEN (1961) did not deduce an increased incidence of carcinoma in 157 cases. This observation was confirmed by EMGE (1953), LARSON (1954) and GREEN (1958–1959). Half of all thecomas are thought to be associated with fibroids (HODGSON, 1945). Meig's syndrome in particular has been described in association with theca-cell tumors.

Estrogen production in the granulosa-theca cell tumor leads to a continuous estrogenic effect on the end organs. This is termed as

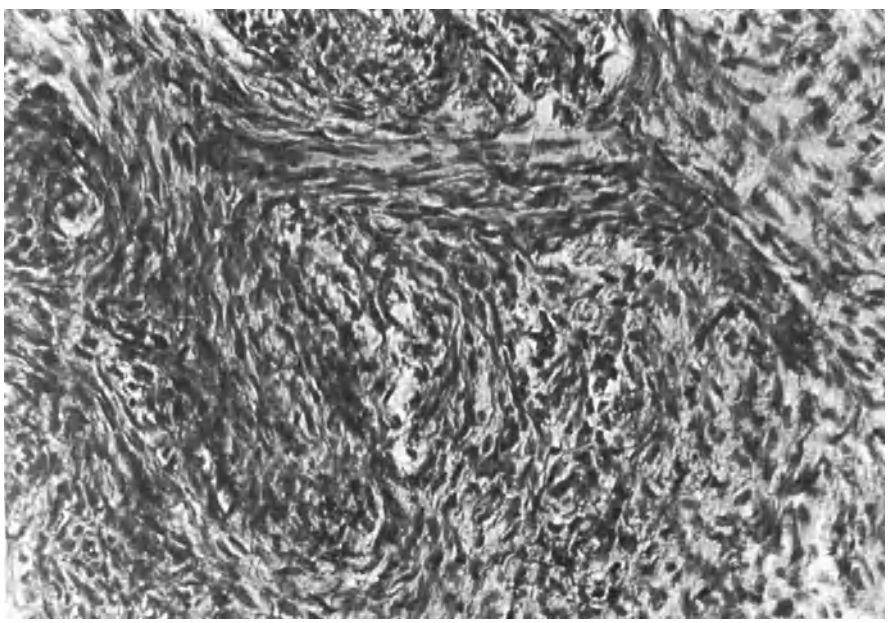


Fig. 77. Theca-cell tumor (LÖFFLER-PRIESEL) of the right ovary in a 68-year-old patient with recurrent uterine bleeding after the menopause

Table 64. Concentrations of estrogens (μg/24 h) in granulosa tumors (KECSÉS, 1963)

	Estrone	Estradiol	Estriol	Total	μg/mg tissue	Urine
Granulosa theca-cell tumor	165.0	0.0	183.0	348.0	24.8	183
Thecoma	0.0	9.2	2.6	11.8	7.3	52
Thecoma	2.0	2.6	0.0	4.6	4.6	50
Granulosa tumor	–	–	–	–	–	64
Anaplastic carcinoma	–	3.0	18.0	21.0	0.3	28
Anaplastic carcinoma	–	10.6	20.0	30.6	0.1	33

estrinism. It is questionable whether partial "hyperestrinism" exists. Hormonal vaginal cytology shows a mere mild to average estrogen effect in the presence of glandular cystic hyperplasia (Fig. 76c) of the endometrium (KÖNIG, 1953).

So-called estrinism results in *precocious pseudopuberty* in the child. This state is characterized by the early development of the genitalia and breasts, the appearance of pubic and axillary hair, anovulatory bleeding before the 9th year, and accelerated increase in height.

During sexual maturity, continuous estrogenic stimulation of the endometrium in the absence of the cyclic influence of progesterone leads to glandular cystic hyperplasia with breakthrough bleeding. This presents clinically as oligomenorrhea or amenorrhea with meno- and metrorrhagia. At the same time, there is swelling of the breasts and hypertrophy of the endometrium.

Analogous changes with bleeding occur during the postmenopause. Climacteric features disappear.

Granulosa-theca cell tumors without the clinical effects described are so rare that the accuracy of the histological diagnosis must be doubted in the absence of the clinical picture (VARANGOT, 1937). Granulosa-theca cell tumors can exceptionally exert an androgenic affect (MORRIS and SCULLY, 1958).

If women with these tumors are young and want children, and the tumor is well-defined and unilateral, treatment is limited to the removal of the diseased ovary. In all other cases, bilateral ovariectomy with hysterectomy is indicated. Infiltration of the surrounding tissues is treated with postoperative radiation. Recurrences also respond very well to radiation.

Some ovarian tumors (cystadenomas, Brenner tumor, ovarian carcinomas) can lead to clinical signs of estrinism. It is assumed that increased amounts of estrogens are produced in the stroma of these tumors (Table 64). Dysfunctional bleeding has been reported in 25% of patients with Brenner tumor, and glandular cystic hypertrophy has been observed in 10% (MORRIS and SCULLY, 1958).

2. Predominantly Androgen-Producing Tumors

Androgenic hormones can be formed in the arrhenoblastoma, gynandroblastoma, hilus-cell tumor, masculinovoblastoma and gonadoblastoma. These are extremely rare tumors, the arrhenoblastoma being the most common of them.

As early as 1900, DAVIS reported the clinical picture of the androgen-active *arrhenoblastoma*. This case involved a 26-year-old woman with defeminization and masculinization, from whom a multilocular cystic ovarian tumor was surgically removed in 1895. In 1905 PICK, and two years later SCHICKELE described the pathoanatomical picture of the so-called *tubular testicular ovarian adenoma*. Endocrine-inactive tumors were present in both cases. R. MEYER (1930) then gave this tumor the name of *arrhenoblastoma*, since he thought the tumor was derived from arrhenocytes, "male-directed" residues of germinal tissue in the medulla and hilus. In Anglo-Saxon literature, the purely descriptive term "Sertoli-Leydig cell tumor" is often preferred since the tumor cells are often similar to immature Sertoli-Leydig cells.

In spite of the aggregation of single observations, the arrhenoblastoma is an uncommon tumor. Thus, up to 1959, HELD and SCHREINER (1959) could only find 187 cases in the literature available to them, and OVERZIER was able to add only 8 more cases up to 1961. JAVERT and FINN (1951) discovered only 3 arrhenoblastomas among 22539 gynecological patients, and not a single one among 74087 obstetric cases. DOCKERTY and MCCARTY (1939), in a resumé of material from the Mayo Clinic between 1900–1936, observed only 4 arrhenoblastomas among 400 solid ovarian tumors. KENT and MCKAY (1960) found only 2 such tumors in y series of 2530 ovarian tumors. We too only found one single case among the histological material from 98663 investigations between 1914–1959 at the Gynecology Clinic of Zurich University.

Only 5% of arrhenoblastomas are bilateral (JAVERT and FINN, 1951). The arrhenoblastoma occurs almost twice as frequently on the right

side (HELD and SCHREINER, 1959). It is most common between the 15th and 30th years. The youngest patient was a girl of $2\frac{1}{2}$ (NOVAK and LONG, 1965).

Observations were originally so rare that the malignancy was not known for a long time and was underestimated. JAVERT and FINN (1951) documented a large sample of patients subjected to prolonged follow-up and found a malignancy index of 22%. This figure was suspected by E. NOVAK even in 1938 and he estimated it even higher on the basis of his wide experience with these tumors (1951). The malignancy index corresponds to that for the granulosa-cell tumor, and many of the recurrences also arise late.

In 80% of the tumors the diameter is between 7–10 cm when they are detected. A few are larger than a man's head. The macroscopic appearance is not characteristic. Arrhenoblastomas can be solid or partly cystic. Yellowish orange areas are frequently encountered. The microscopic picture also varies widely, three main types being distinguishable (R. MEYER, 1930): a highly differentiated tubular form, an intermediary form and an undifferentiated sarcoma-like form. The large eosinophilic interstitial cells containing lipoids are the most interesting functional structure. They probably correspond morphologically and functionally to the Leydig interstitial cells of the testis, and they must be assumed to be the site of formation of the androgenic hormones (Fig. 78a). According to TEILUM (1952), neoplastic proliferation alone of these interstitial cells leads to the formation of the hypernephroid ovarian tumor he considers as an extreme variety of the so-called androblastoma, and he also classifies the arrhenoblastoma in this group.

The clinical picture is characterized by defeminization with subsequent virilism. Oligo- or amenorrhea arises. Meno-metrorrhagia occasionally occurs temporarily. Breasts, endometrium, myometrium and vaginal epithelium

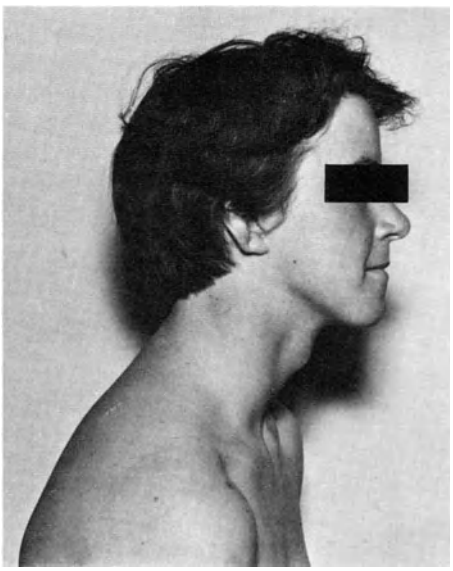
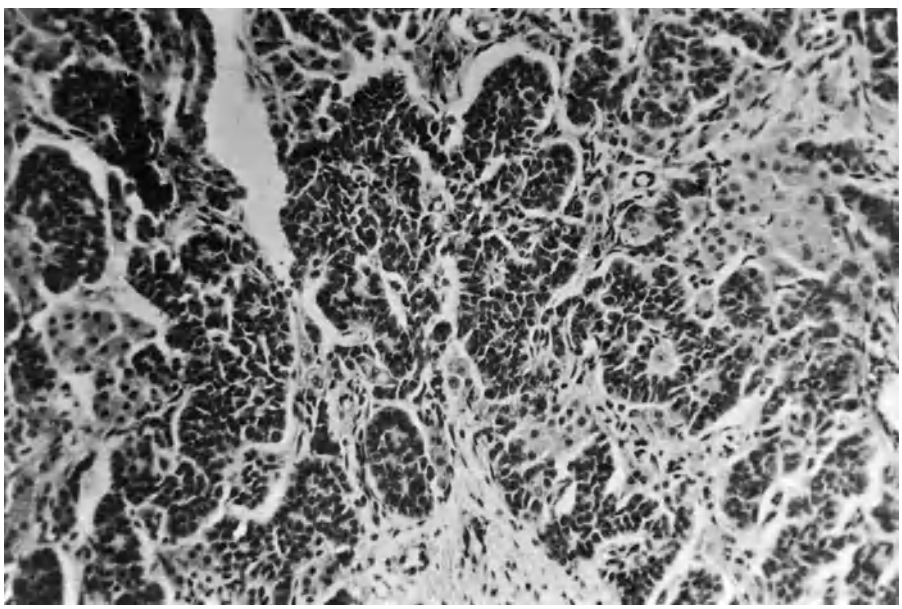


Fig. 78b



a

Fig. 78a and b. Mixed-type arrhenoblastoma (a) of the right ovary in a 16-year-old girl with virilization. Prominentia laryngea (b)

become atrophic (Fig. 78a). Secondary sexual characteristics regress. Hirsutism and acne arise, the clitoris becomes hypertrophic and frontal baldness appears. The voice becomes deeper and an Adam's apple (Fig. 78b) may be formed. The body stature assumes the masculine form.

Removal of the tumor causes refeminization, but hypertrophy of the clitoris and the deep voice may persist. Recurrences produce defeminization and virilism again (WAGNER, 1930; MEIXNER, 1958).

Chemical investigations of the tumor tissue have allowed the deduction that considerable amounts of androgens are produced by endocrine-active arrhenoblastomas (Table 65a–c). Judging from the clinical picture, these amounts probably correspond to those produced in the male. The heterosexual action of the arrhenoblastoma is often in marked contrast to the excretion of the 17-ketosteroids, which is at the upper normal limit or only slightly elevated in the majority of cases (Table 66). There are, however, numerous reports (HELD and SCHREINER, 1959) of cases where urinary 17-ketosteroids are definitely increased, but these values have never been as high as those found in the presence of tumors of hyperplasia of the adrenal cortex.

Table 65a. Steroids[a] in an arrhenoblastoma in a 24-year-old woman with defeminization and virilization; excretion of 17-ketosteroids within normal range (OBER, 1959)

	µg/kg tissue
Testosterone	1280
Δ_4-Androstendione	240
Progesterone	930
Δ_4-pregnen-3-on,20α-ol	360
17α-OH progesterone	1110

[a] After ANLIKER (1956).

Table 65b. Steroids in a 145 g intermediate arrhenoblastoma on the right ovary of a 26-year-old patient with defeminization and virilization; excretion of 17-ketosteroids 13.9 mg/day (WINZELER, 1957)

	µg/kg tissue
Testosterone	92
Δ_4-Androstendione	370
Androsterone	61
Progesterone	31
Corticosteroids	0
Estrogens	0

Table 65c. Testosterone in different tissues (RÜTTNER, 1957)

	µg/kg tissue
Bovine testis	100
Porcine testis	30
Equine testis	200
Metastases of an adrenocortical tumor	45–55

Table 65d. Steroids[a] in an intermediate arrhenoblastoma weighing approximately 80 g on the right ovary of a 16-year-old girl; it had led to defeminization and virilization. Excretion of 17-ketosteroids 32.6 mg/day (HELD, 1959)

	µg/kg tissue
Testosterone	37–75
Δ_4-Androstendione	262–300
Androsterone	375
Progesterone	150
Corticosteroids	0
Estrogens	0

[a] After ANLIKER (1956).

The defeminization and virilism occurring with the arrhenoblastoma must therefore be considered as being mainly a long-term action of relatively slightly increased testosterone production over a long period of time. This assumption is confirmed by the observation that pituitary gonadotropins are reduced but not fully suppressed. With the exception of LOBOTSKY's case (1954), the pregnanediol-pregnanetriol complex is negative, despite the considerable amounts of progesterone which can be demonstrated chemically in the tumor (Table 65).

Not all arrhenoblastomas are endocrine-active. Thirteen of 111 cases in "The American Ovarian Tumor Registry" showed no androgenic action (NOVAK and LONG, 1965). Only about half the differentiated tumors lead to the symptomatology described. About 75% of intermediary tumors are endocrine-active, whereas nearly all undifferentiated types are androgen-active (KRAUS, 1967).

Gynandroblastomas (R. MEYER, 1930) are very uncommon and are mixed tumors consisting of granulosa-cell tumors and arrhenoblastomas. Accordingly, they have an androgenic and an estrogenic effect, but the heterosexual differentiation caused by the androgens dominates the clinical picture (EMIG, 1959). Only 14 certain cases have so far been described (HUGHESDON and FRAZER, 1953; KISTNER, 1964). The diameter of the tumor was found to vary between 1–20 cm. *Hilus(Leydig)-cell tumors* (sympathicotropic-cell tumors) are just as rare (NOVAK and MATTINGLEY, 1960). Characteristic histological findings are Reinke's crystalloids and the arrangement of tumor cells in the region of amyelinic nerve fibers. These tumors are solid, yellowish, with well-demarcated growths in the hilus of the ovary. They seldom have a diameter of more than 5 cm and they are benign. In contrast to arrhenoblastomas they arise mainly during the climacteric or postmenopause. As with arrhenoblastomas, the 17-ketosteroid excretion is at the normal upper limit or slightly elevated (SCULLY, 1963).

Table 66. Excretion of 17-ketosteroids and pregnandiol in the presence of arrhenoblastoma (HELD, 1959)

	Age	17-Ketosteroids (mg/24 h)		Other hormone estimations
		Preop.	Postop.	
LOBOTSKY, J. (1954)	16	12–90	–	Androgens 5–35 IU Pregnand. 21–24 mg
MALLORY, T. B. (1939)	29	6.3–8.5	–	
MALLORY, T. B. (1941)	53	3.9–5.5	–	
MEIXNER, H. (1957)	20	21	–	
NELSON, H. B. (1957)	18	11.4	–	
NOKES, J. M., and CLAIBORNE, H. A. (1956)	a) 31	a) 13–16.5	–	
	b) 36	b) 9–10.5	–	
	c) 62	c) "normal"	–	
	d) 34	d) 12.5	–	
NORDEN, G., and DAHLBERG, B. (1956)	65	3.3	7.3	
PLATE, W. P. (1947)	27	16.9	2.6–5.2	
RILEY, CH., and MURPHY, G. H. (1951)	18	6.7	–	Pregnandiol neg.
SMILEY, I. (1953)	22	64	11.2	
THOMAS, C. Y. (1952)	$11\frac{3}{4}$	7.2	7.8	
WACHS, CH. S., and WEBER, L. L. (1951)	21	10.4	9.3	
WINZELER, H. (1957)	26	13.9	–	
COHEN, M. (1958)	38	14.1–19.8 (average 16.1)	average 6	
EVANS, P. R. C., and JONES, R. O. (1955)	15	7.8–9	"normal"	
FLANNERY, W. E. (1950)	$13\frac{1}{2}$	6.3–7.4	–	
FRASER, R. W. (1941)	30	5.3–8.5	2.4–3.9	
GILBERT-DREYFUS, J. (1957)	17	18.7–22	6–11.6	Corticosteroids 2–5 mg/24 h
GREENBLATT, R. B. (1955)	?	31.7–37.6	–	
HELD, E., and SCHREINER, W. E. (1959)	16	32.6	2.3–7.2	Corticosteroids 5.0 preop. 4.1 postop. Pregnand. neg. preop.
JONES, G. S., and EVERETT, H. S. (1946)	26	36–56	11	Pregnandiol neg.
JONES, H. W., and SCOTT, W. W. (1958)	23	"normal"	–	
IVERSON, L. (1947)	61	10.9	8.8	
KING, J. M. (1955)	28	59	12.1	
LEVERTON, J. S. (1955)	27	31.4	4.7–10.7	
STABLER (1955)	?	4.5	–	

So-called *virilizing lipoid-cell tumors* (BARZILAI, 1943) are somewhat more common (masculinovoblastoma, adrenal remnant tumor, hypernephroid tumor, diffuse androblastoma). As can be deduced from the large number of names, this is a heterogeneous group; hilus-cell tumors, thecomas or adrenal remnant tumors may be present. Like granulosa cell tumors and arrhenoblastomas, 95% are unilateral. They can also cause defeminization and virilism. Adrenal remnant tumors can also give rise to signs of Cushing's syndrome (MORRIS and SCULLY, 1958). Excretion of 17-ketosteroids is rather more frequently increased than with arrhenoblastomas (MORRIS and SCULLY, 1958). The β-fraction of this excretion may be greatly increased with adrenal rest tumors and values as high as those with adrenal tumors may be reached (BAUER and KARL, 1952; DEVIS, 1956). Lipoid-cell tumors are yellowish brown, solid, partly cystic, well-defined, and arise almost twice as frequently before the menopause as during the postmenopause. They consist mainly of large, round or polygonal cells with rich eosinophilic or clear, vacuolized cytoplasm. Incubation studies have shown that there is a metabolic block where testosterone and androstendione cannot be aromatized by the tumor cells (BRYSON, 1962). Formation of metastases with a fatal outcome has frequently been observed (MORRIS and SCULLY, 1958). The malignancy index is similar to that of the granulosa-cell tumor and arrhenoblastoma and is given as 21% (PEDOWITZ and POMERANCE, 1962).

The *gonadoblastoma* (MORRIS and SCULLY, 1958; SCULLY, 1963) is very rare, and also leads to defeminization and virilism. It consists of immature germinal cells, Sertoli (granulosa) and Leydig (theca) cells. The histological picture is similar to that of the embryonic gonad, hence the name. The tumor has been described most frequently in chromatin-negative patients with female appearance, all of whom had a uterus and tubes. Testes or ovaries have never been found. The tumors are solid. Multiple calcifications are characteristic histological findings and are also conspicuous on X-rays. The few estimations of 17-ketosteroids in the urine have shown normal or slightly elevated values.

The *therapeutic procedure* in all cases of endocrine-active tumors is determined by the age of the patient, and by the local findings and histology. In patients over 40, hysterectomy with bilateral removal of the ovaries is indicated; this is the procedure we adopt for all ovarian tumors. In patients under 40, if the tumor is strictly restricted to one ovary, the capsule is intact and histology can exclude a sarcomatous growth, surgery can be limited to unilateral ovariectomy. If there is the slightest suspicion of malignancy, hysterectomy with bilateral ovariectomy should be performed and followed by radiation.

Apart from the ovarian tumors mentioned, pseudomycincystomas, adenocarcinomas, Brenner tumors, Krukenberg tumors and other ovarian metastases may also be associated with virilism (PLOTZ, 1966). All these tumors show luteinization. The few measurements of 17-ketosteroids excretion so far performed show normal levels (SCULLY, 1963).

Differentiation between the androgen-producing ovarian tumor and tumors and hyperplasia of the adrenal usually presents no difficulties. In the former type, excretion of 17-ketosteroids is often grossly elevated. With the adrenal tumor, the excretion is occasionally over 100 mg/day, the β-fraction being over 50%. In contrast, excretion of androsterone and etiocholanolone is fairly high in the presence of ovarian tumors. Pregnanetriol excretion is increased in adrenocortical hyperplasia, whereas this is not so with adrenogenic ovarian tumors. Prepubertal development of clinical symptoms is indicative of an adrenal tumor or hyperplasia. Since the endocrine-active ovarian tumor can only be palpated in 60% of cases, it can often only be confirmed by means of culdoscopy or laparoscopy. Retropneumoperitoneum and intravenous urogram may be helpful in differentiation. There may be difficulty in differentiating between the ovarian adrenal remnant tumor and the adrenocortical tumor, since the clinical picture and hormonal analytical findings may be identical. Urogram, culdoscopy, laparoscopy and retropneumoperitoneum are further methods of diagnostic investigation. If symptoms such as hypertension, polycythemia, reduced glucose tolerance and striae arise, the possibility of Cushing's syndrome due to hypothalamo-hypophyseal factors must be considered. If this is present there is no virilism and 17-ketosteroid excretion is not greatly elevated.

In the absence of a palpable ovarian tumor, it is difficult to differentiate between borderline adrenal hyperplasia (postpubertal increased formation of adrenal androgens), "idiopathic hirsutism" and the syndrome of polycystic ovaries, which can all give rise to virilism in exceptional cases. Routine measurement of steroid excretion does not help. French authors (NETTER, 1961; MAUVAIS-JARVIS and BAULIEU, 1961; BAULIEU, 1963; JAYLE, 1967) have recommended the combined dexamethasone/HCG test for differentiation of ovarian androgen formation from adrenal androgen formation. This test is performed and interpreted as follows: the patient receives 3 mg dexamethasone daily for 12 days, and in addition 5000 IU HCG on the 6th, 7th and 8th days. Steroid excretion (17-ketosteroids, androsterone, etiocholanolone and dehydroepiandrosterone) are measured before the test and on the 5th and 7th days. In the healthy patient, the 17-ketosteroids fall to less than 2 mg/day. In the presence of increased formation of ovarian androgens, 17-ketosteroid excretion is more than 3 mg/day, and HCG stimulation should produce another rise. Clinical experience with this functional test is still too limited to permit definite assessment of its significance. Results of other investigators (PERLOFF and JACOBSON, 1963; SOHVAL and SOFFER, 1951) appear to restrict the value of this test considerably (cf. Stein-Leventhal syndrome, p. 609).

Rapid progression of the defeminization and virilism is indicative of the presence of an androgen-producing tumor and makes idiopathic hirsutism, postpubertal increased formation of androgens and Stein-Leventhal syndrome unlikely. Culdoscopy and laparoscopy will help in further differentiation in these cases also.

3. Chorioepithelioma

A primary ovarian chorioepithelioma arises from a teratoblastoma (Fig. 79) or from the trophoblastic tissue of an ovarian pregnancy. A secondary chorioepithelioma usually originates in a uterine chorioepithelioma. The primary ovarian tumor is rare, and extremely malignant. Gestational tumors arising in an ovarian pregnancy will respond to cytostatic therapy (Methotrexat, Actinomycin-D, Chlorambucil), whereas those arising in a teratoblastoma are without exception fatal. It may cause precocious pseudopuberty when it occurs before menarche (WILKINS, 1965), probably because of the increased production of estrogens by the ovaries, which is caused in turn by the stimulation of chorionic gonadotropin. Correspondingly, there may be multiple granulosa and theca lutein cysts in the intact ovary. After menarche, acyclic bleeding and signs of a pregnancy are the dominant clinical features. Biological and immunological pregnancy tests are positive.

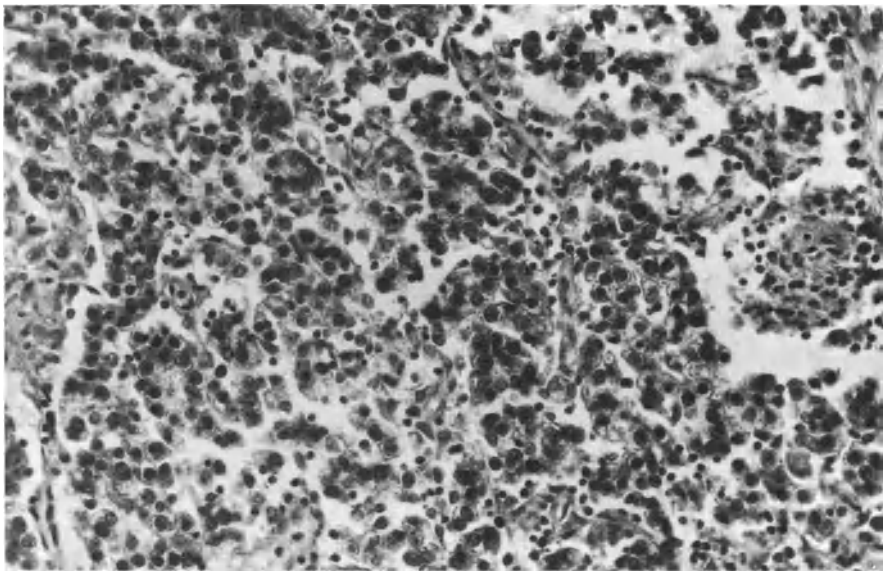


Fig. 79. Teratoma embryonale of the left ovary progressing to chorial carcinoma in a 15-year-old girl. Implantation metastases in the rectouterine pouch. 30000 IU HCG/liter urine; 39.2 mg estriol/24 h

4. Struma Ovarii

Thyroid tissue occurring as a component of a dermoid cyst (teratoma adultum ovarii) is by no means an uncommon finding. Exceptionally, the thyroid tissue predominates to such an extent that a so-called struma ovarii develops. In about 20% of these cases, no other teratomatous tissue can be detected microscopically (SAILER, 1943). Thyroid tissue tumors are bilateral in less than 5% of cases. Macroscopic and microscopic findings correspond largely to those in a nodular colloid goiter. The histological picture usually shows little glandular tissue, and characteristic lobular structures. Adenomatous areas occasionally arise within the colloid goiter (MÜLLER, 1961). About 12% of struma ovarii exhibit carcinomatous regions, and metastases are found in about half these cases (MÜLLER, 1946). Clinical signs of thyreotoxicosis are seen in 10% of cases (SMITH, 1946), but may disappear after removal of the tumor (MÜLLER, 1961). The iodine content only exceptionally corresponds to that of a normal adult thyroid gland, usually being much reduced or completely absent. Dystopic ovarian thyroid tissue can be demonstrated by the radioiodine test in these cases (MÜLLER, 1954).

References

Historical Dates

ADAM, N. K., DANIELLI, J. F., DODDS, E. C., KING, H., MARRIAN, G. F., PARKES, A. S., ROSENHEIM, O.: Nomenclature of oestrin group. Nature (Lond.) **132**, 205 (1933).

ADLER, L.: Zur Physiologie und Pathologie der Ovarialfunktion. Arch. Gynäk. **95**, 349 (1910).

ALLEN, E., DOISY, E. A.: Ovarian hormone: preliminary report on its localization, extraction and partial purification, and action in test animals. J. Amer. med. Ass. **81**, 819 (1923).

ASCHHEIM, S.: Diskussionsbemerkung. Berliner gynäkologische Gesellschaft 14. 12. 28. Z. Geburtsh. Gynäk. **45**, 371 (1929).

— ZONDEK, B.: Hypophysenvorderlappenhormon und Ovarialhormon im Harn von Schwangeren. Klin. Wschr. **6**, 1322 (1927).

BAER v., C. E.: De ovi mammalium et hominis genesi. Epistola ad Academ. Imper. Petropol. Lipsiae 1827.

BREUER, H.: Vorkommen und Charakterisierung von Steroiden in Nebenhodengewebe, Vesiculardrüsensekret und Nebenhodenspermatozoen. Biochem. Z. **327**, 6 (1955).

BUESS, H.: Die historischen Grundlagen der wissenschaftlichen Gynäkologie in Tabellenform. Gynaecologia (Basel) **162**, 453 (1966).

BUTENANDT, A.: Über Progynon, ein kristallisiertes weibliches Hormon. Naturwissenschaften **17**, 879 (1929).

— Über physikalische und chemische Eigenschaften des kristallisierten Follikelhormons. Untersuchungen über das weibliche Sexualhormon. Hoppe-Seylers Z. physiol. Chem. **191**, 140 (1930).

— WESTPHAL, U.: Zur Isolierung und Charakterisierung des Corpus-luteum-Hormons. Ber. dtsch. chem. Ges. **67**, 1440 (1934).

CLAUBERG, C.: Zur Physiologie und Pathologie der Sexualhormone, im Besonderen des Hormons des Corpus luteum, 1. Mitteilung. Der biologische Test für das Luteumhormon (des spezifischen Hormons des Corpus luteum) am infantilen Kaninchen. Zbl. Gynäk. **54**, 2757 (1930).

CLAUS, P. E.: Separation of anterior-lobe substances and study of their individual effects. Physiol. Zool. **4**, 36 (1931).

COHEN, S. L., MARRIAN, G. F.: The application of the Kober test to the quantitative estimation of oestrone and oestriol in human pregnancy urine. Biochem. J. **28**, 1603 (1934).

CORNER, G. W., ALLEN, W. M.: Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of Corpus luteum. Amer. J. Physiol. **88**, 326 (1929).

DJERASSI, C., MIRAMONTES, L., ROSENKRANZ, G., SONDHEIMER, F.: Synthesis of 19-nor-17-alpha-ethinyltestosterone and 19-nor-17-alpha-methyltestosterone. J. Amer. chem. Soc. **76**, 4092 (1954).

DOISY, E. A., VELER, C. D., THAYER, S. A.: Folliculin from urine of pregnant women. Amer. J. Physiol. **90**, 329 (1929).

EVANS, H. M., LONG, J. A.: The effect of feeding the anterior lobe of the hypophysis on the oestrous cycle of the rat. Cited by HAMBURGER, C. In: Gonadotropins. Ciba Found. Study Group vol. 22, p. 3. London: Churchill 1965.

FELLNER, O. O.: Experimentell erzeugte Wachstumsveränderungen am weiblichen Genitale der Kaninchen. Zbl. allg. Path. path. Anat. **23**, 673 (1912).

— Experimentelle Untersuchungen über die Wirkung von Gewebsextrakten aus der Plazenta und den weiblichen Sexualorganen auf das Genitale. Arch. Gynäk. **100**, 641 (1913).

FEVOLD, H. L., HISAW, F. L., LEONHARD, S. L.: The gonad stimulating and the luteinizing hormone of the anterior lobe of the hypophysis. Amer. J. Physiol. **97**, 291 (1931).

FISCHER, I.: Geschichte der Gynaekologie. In: Biologie und Pathologie des Weibes, HALBAN, J., L. SEITZ, Eds., Bd. I. Berlin: Urban & Schwarzenberg 1924.

— Historischer Rückblick über die Leistungen des 19. Jahrhunderts auf dem Gebiete der Geburtshilfe und Gynäkologie. In: Biologie und Pathologie des Weibes, HALBAN, J., L. SEITZ, Eds., Bd. 8, 3. Teil. Berlin: Urban & Schwarzenberg 1929.

FRANK, R. T.: The corpus luteum as the source of the follicular hormone. Surg. Gynec. Obstet. **42**, 572 (1926).

FRÄNKEL, L.: Versuche über den Einfluß der Ovarien auf die Insertion des Eies. Verh. dtsch. Ges. Gynäk. **9**, 571 (1901).

— Das zeitliche Verhalten von Ovulation und Menstruation. Zbl. Gynäk. **35**, 1591 (1911).

HALBAN, J.: Über den Einfluß der Ovarien auf die Entwicklung des Genitales. Mschr. Geburtsh. Gynäk. **12**, 496 (1900).

HARRIS, G. W.: The neural control of the pituitary gland. London: Arnold 1955.

HERMANN, E.: Über eine wirksame Substanz im Eierstock und in der Placenta. Mschr. Geburtsh. Gynäk. **41**, 1 (1915).

HERTZ, R., TULLNER, W., RAFFELT, E.: Progestational activity of orally administered 17-alpha-ethinyl-19-nortestosterone. Endocrinology **54**, 228 (1954).

HOHLWEG, W., JUNKMANN, K.: Die hormonal-nervöse Regulierung der Funktion des Hypophysenvorderlappens. Klin. Wschr. **11**, 321 (1932).

INHOFFEN, H. H.: Übergang von Sterinen in aromatische Verbindungen. Angew. Chem. **53**, 471 (1940).

— HOHLWEG, W.: Neue per os-wirksame weibliche Keimdrüsenhormonderivate: 17-Aethinyl-oestradiol und Pregnen-in-on-3-ol-17. Naturwissenschaften **26**, 96 (1938).

KAUFMANN, C.: Umwandlung der Uterusschleimhaut einer kastrierten Frau aus dem atrophischen Stadium in das der sekretorischen Funktion durch Ovarialhormone. Zbl. Gynäk. **56**, 2058 (1932).

KNAUER, E.: Einige Versuche von Ovarialtransplantation an Kaninchen. Zbl. Gynäk. **20**, 524 (1896).

KNAUS, H.: Zur Physiologie des Corpus luteum. Arch. Gynäk. **138**, 201 (1929); **140**, 181 (1930); **141**, 374, 395 (1930).

KUNDRAT, H., ENGELMANN, G. J.: Untersuchungen über die Uterusschleimhaut. Wien. med. Jahrb. 136 (1873).

MARKEE, J. E., SAWYER, CH. H., HOLLINS-HEAD, W. H.: Activation of the anterior hypophysis by electrical stimulation in the rabbit. Endocrinology **38**, 345 (1946).

MARRIAN, G. F.: The chemistry of oestrin. I. Preparation from urine and separation from an unidentified solid alcohol. Biochem. J. **23**, 1090 (1929).

— BAULD, W. S.: Isolation of 16-epioestriol from urine of pregnant women. Biochem. J. **59**, 136 (1955).

MARSHALL, F. H. A., JOLLY, W. A.: Contributions to the physiology of mammalian reproduction. Part I.—The oestrus cycle in the dog. Part II.—The ovary as an organ of internal secretion. Proc. roy. Soc. B **76**, 395 (1905).

MCCORQUODALE, D. W., THAYER, S. A., DOISY, E. A.: The crystalline ovarian follicular hormone. Proc. Soc. exp. Biol. (N. Y.) **32**, 1182 (1935).

MEYER, R.: Über die Beziehung der Eizelle und des befruchteten Eies zum Follikelapparat, sowie des Corpus luteum zur Menstruation. Arch. Gynäk. **100**, 1 (1913).

PHILIPP, E.: Sexualhormone, Placenta und Neugeborenes. Zbl. Gynäk. **53**, 2386 (1929).

POPA, S. T., FIELDING, U.: A portal circulation from the pituitary to the hypothalamic region. J. Anat. (Lond.) **65**, 88 (1930).

PRÉNANT, L. A.: Sur la valeur morphologique, sur l'action physiologique et thérapeutique possible du corps jaune. Rev. méd. Nancy **30**, 385 (1898).

RÉGIS, E.: Cas de folie consécutive à une ovaro-salpingectomie. Nouv. Arch. Obstet. **8**, 556 (1893).

RICCI, J. V.: One hundred years of gynecology. Philadelphia: Blakiston Comp. 1945.

SAFFRAN, M., SCHALLY, A. V.: Release of corticotrophin by anterior pituitary tissue *in vitro*. Canad. J. Biochem. **33**, 408 (1955).

SCHRÖDER, R.: Der normale Menstruationszyklus. Berlin: A. Hirschwald 1913.

— Über die zeitlichen Beziehungen der Ovulation und Menstruation. Arch. Gynäk. **101**, 1 (1914).

SIGISMUND, R.: Ideen über das Wesen der Menstruation. Berl. klin. Wschr. **8**, 624 (1871).

SLOTTA, W., RUSCHNIG, W., FELS, W.: Reindarstellung der Hormone aus dem Corpus luteum. Ber. dtsch. chem. Ges. **67**, 1270 (1934).

SMITH, P. E.: Hastening development of female genital system by daily homoplastic pituitary transplants. Proc. Soc. exp. Biol. (N. Y.) **24**, 131 (1926).

STOCKARD, C. R., PAPANICOLAOU, G. N.: The existence of a typical oestrous cycle in the guinea-pig; with a study of its histological and psychological changes. Amer. J. Anat. **22**, 225 (1917).

ZANDER, J., FORBES, T. R., MÜNSTERMANN, A. M. VON, NEHER, R. J.: Δ^4-3-Ketopregnene-20α-ol and Δ^4-3-Ketopregnene-20β-ol, two naturally ocurring metabolites of progesterone. J. clin. Endocr. **18**, 337 (1958).

ZONDEK, B., ASCHHEIM, S.: Das Hormon des Hypophysenvorderlappens. Darstellung, chemische Eigenschaften, biologische Wirkungen. Klin. Wschr. **18**, 831 (1928).

Anatomy

BARGMANN, W.: Histologie und mikroskopische Anatomie des Menschen. Stuttgart: Thieme 1956.

BLANDAU, R. J.: The mechanism of ovulation. In: Ovulation, GREENBLATT, R. B., Ed. Philadelphia-Toronto: Lippincott 1966.

— Anatomy of ovulation. Clin. Obstet. Gynec. **10**, 347 (1967).

— WARRICK, E., RUMERY, R. E.: *In vitro* cultivation of fetal mouse ovaries. Fertil. Steril. **16**, 705 (1965).

BLOCK, E.: Quantitative morphological investigations of the follicular system in women. Variations at different ages. Act. anat. (Basel) **14**, 108 (1952).

CORNER, G. W.: Events of primate ovarian cycle (Huxley lecture). Brit. med. J. **1952 II**, 403.

— The histological dating of the human corpus luteum of menstruation. Amer. J. Anat. **98**, 377 (1956).

DANTCHAKOFF, V.: Von der entodermalen Wanderzelle bis zur Urkeimzelle in der Gonade. Z. Zellforsch. **13** (1931).

DEANE, H. W.: Histochemical observations on the ovary and the oviduct of the albino rat during the oestrous cycle. Amer. J. Anat. **91**, 363 (1952).

ERSKINE, C.A., RANNIE, I.: Bilateral ovarian aplasia. Arch. Path. **42**, 381 (1946).

GREEP, R.O.: Histology, Histochemistry and ultrastructure of adult ovary. In: The ovary, GRADY, H.G., and D.E. SMITH, Eds. Baltimore: Williams & Wilkins 1963.

HARRISON, R.J.: The structure of the ovary: C. Mammals. In: The ovary, vol. I, S. ZUCKERMAN, Ed. New York: Academic Press 1962.

HARTMAN, C.G.: Science and the safe period. Baltimore: Williams & Wilkins 1962.

HISAW, F.L.: Development of the graafian follicle and ovulation. Physiol. Rev. **27**, 95 (1947).

IKONEN, M., NIEMI, M., PESONEN, S., TIMONEN, S.: Histochemical localization of four dehydrogenase systems in human ovary during the menstrual cycle. Acta endocr. (Kbh.) **38**, 293 (1961).

ISRAEL, S.L.: Menstrual disorders and sterility. New York: Hoeber 1967.

JUNG, G., KIDES, E.: Proteolytische Fermente im Ovargewebe. Arch. Gynäk. **192**, 151 (1959).

LAURITZEN, CH.: Das Klimakterium. Med. Mitt. (Schering) **4**, 2 (1968).

MERRILL, J.: Ovarian hilus cells. Amer. J. Obstet. Gynec. **78**, 1258 (1959).

MEYER, R.: Über Corpus-luteum-Bildung beim Menschen. Zbl. Gynäk. **35**, 1549 (1911).

— Über Corpus-luteum-Bildung beim Menschen. Arch. Gynäk. **93**, 354 (1911).

— Über die Beziehung der Eizelle und des befruchteten Eies zum Follikelapparat sowie des Corpus luteum zur Menstruation. Arch. Gynäk. **100**, 1 (1913).

MÖLLENDORFF, W. v.: Handbuch der mikroskopischen Anatomie des Menschen. Berlin: Springer 1930.

MORICARD, R., GOTHIE, S.: Dissociation des cellules de la granulosa et problème d'un mécanisme diastatique dans la rupture du follicle ovarien de lapine. C.R. Soc. Biol. (Paris) **140**, 249 (1946).

OHNO, S., SMITH, J.B.: Role of fetal follicular cells in meiosis of mammalian oocytes. Cytogenetics **3**, 324 (1964).

OVERZIER, C.: Echter Agonadismus. In: Die Intersexualität, OVERZIER, C., Ed. Stuttgart: Thieme 1961.

SCHRÖDER, R.: Weibliche Genitalorgane. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. 7, 1. Teil. Berlin: Springer 1930.

SHETTLES, L.B.: Ovum humanum. Munich-Berlin: Urban & Schwarzenberg 1960.

STAEMMLER, H.J.: Die gestörte Regelung der Ovarialfunktion. Berlin-Göttingen-Heidelberg: Springer 1964.

STIEVE, H.: Über Follikelreifung, Gelbkörperbildung und den Zeitpunkt der Befruchtung beim Menschen. Z. mikr.-anat. Forsch. **53**, 467 (1943), Leipzig: Akadem. Verlagsgesellschaft Becker & Erler 1943.

— Der Einfluß des Nervensystems auf Bau und Tätigkeit der Geschlechtsorgane des Menschen. Stuttgart: Thieme 1952.

— Normale Anatomie der endokrinen Drüsen und endokrine Regulation. In: Lehrbuch der speziellen pathologischen Anatomie, KAUFMANN, E., revised by STAEMMLER, M., Bd. I/2, S. 1285. Berlin: W. de Gruyter 1956.

VALDES-DAPENA, M.: Normal ovary of childhood. Ann. N.Y. Acad. Sci. **142**, 597 (1967).

WALTON, A., HAMMOND, J.: Observation on ovulation in the rabbit. Brit. J. exp. Biol. **6**, 190 (1928).

WATZKA, M.: Weibliche Genitalorgane. Das Ovarium. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. VII/3., MÖLLENDORF, W.v. and W. BARGMANN, Eds. Berlin-Göttingen-Heidelberg: Springer 1957.

ZUCKERMAN, S.: The number of oocytes in the mature ovary. Recent Progr. Hormone Res. **6**, 63 (1951).

— The law of follicular constancy. Acta physiol. lat.-amer. **3**, 198 (1953).

The Physiology of Ovarian Function

ALBRECHTSEN, O.K.: The fibrinolytic activity of the human endometrium. Acta endocr. (Kbh.) **23**, 207 (1956).

ALLEN, E., DOISY, E.A.: An ovarian hormone: a preliminary report on its localization, extraction and partial purification, and action in test animals. J. Amer. med. Ass. **81**, 819 (1923).

ANDREOLI, C., PORTA, DELLA M.: Cyclic changes in female saliva. J. clin. Endocr. **17**, 913 (1957).

AREY, L.B.: The degree of normal menstrual irregularity. An analysis of 20000 calendar records from 1500 individuals. Amer. J. Obstet. Gynec. **37**, 12 (1939).

ARTNER, J.: Die vegetative Steuerung des Zyklus. Arch. Gynäk. **185**, 85 (1954).

— Die rhythmischen Schwankungen im vegetativen System im Verlauf des Zyklus. Geburtsh. Frauenheilk. **14**, 677 (1954).

— Vegetative Ausgangslage und Zyklus. Arch. Gynäk. **192**, 379 (1959).

— Die Bedeutung der vegetativen Untersuchung in der gynäkologischen Endokrinologie. Fortsch. Geburtsh. Gynäk. **10**, 1 (1960).

ASHLEY-MONTAGU, M.F.: Adolescent sterility. Springfield (Ill.): Ch.C. Thomas 1946.

ASPLUND, J.: Uterine cervix and isthmus under normal and pathological conditions; clinical and roentgenological study. Acta radiol. (Stockh.) **91**, Suppl. 3 (1952).

AYDAR, C.K., GREENBLATT, R.B.: Clinical observations on a new progestational agent "Allyl-estrenol". Acta endocr. (Kbh.) **38**, 419 (1961).

BALDWIN, R.M., WHALLEY, P.J., PRITCHARD, J.A.: Measurement of menstrual blood loss. Amer. J. Obstet. Gynec. **81**, 739 (1961).

BARER, A.P., FOWLER, W.M.: The blood loss during normal menstruation. Amer. J. Obstet. Gynec. **31**, 979 (1936).

BARTELMEZ, G.W.: The human uterine mucous membrane during menstruation. Amer. J. Obstet. Gynec. **21**, 623 (1931).

— Histological studies on the menstruating mucous membrane of the human uterus. Contr. Embryol. Carneg. Instn **24**, 141 (1933).

— Menstruation. Physiol. Rev. **17**, 28 (1937).

— The phases of the menstrual cycle and their interpretation in terms of the pregnancy cycle. Amer. J. Obstet. Gynec. **74**, 931 (1957).

BEER, C.T., GALLAGHER, T.F.: Excretion of estrogen metabolites by humans. I. The fate of small doses of estrone and estradiol-17β. J. biol. Chem. **214**, 335 (1955).

BEHRENS, H.: Die Variationsmöglichkeiten im Aufbau der Uterusschleimhaut in den einzelnen Phasen des mensuellen Zyklus. Leipzig: VEB, Thieme 1953.

BELLER, F.K., GOEBELSMANN, U., DOUGLAS, G.W., JOHNSON, A.: The fibrinolytic system during the menstrual cycle. Obstet. and Gynec. **23**, 12 (1964).

— MATSI, M., EPSTEIN, M.D.: Incoagulability of menstrual blood. Amer. J. Obstet. Gynec. **102**, 1121 (1968).

BISHOP, P.M.F., RICHARDS, N.A., PERRY, W.L.M.: Stilboestrol sulphate, oestrone and equilin. Further observations on the potency and clinical assessment of oestrogens. Lancet **1951 I**, 818.

BJÖRNSON, J.: Über die normale Variabilität des menschlichen Menstruationsintervalles. Arch. Gynäk. **164**, 619 (1937).

BOSCHANN, H.W.: Praktische Zytologie. Berlin: W. de Gruyter 1960.

BOSCOTT, R.J.: The chemistry and biochemistry of natural and artificial oestrogens. The chemistry of progesterone and relaxin. In: The ovary, vol. II, S. ZUCKERMAN, Ed. New York, London: Academic Press 1962.

BROWN, J.B.: Urinary excretion of oestrogen during the menstrual cycle. Lancet **1955 I**, 320.

— A chemical method for the determination of oestriol, oestrone and oestradiol in human urine. Biochem. J. **60**, 185 (1955).
— The relationship between urinary oestrogens and oestrogens produced in the body. J. Endocr. **16**, 202 (1957).
— The metabolism of oestrogens and the measurement of the excretory products in the urine. J. Obstet. Gynaec. Brit. Emp. **66**, 795 (1959).
— Oestrogens in the human female. In: Modern trends in human reproductive physiology, vol. I., CAREY, H.M., p. 49. London: Butterworths 1963.
— BULBROOK, R.D., GREENWOOD, F.C.: An additional purification step for a method for estimating oestriol oestrone and oestradiol-17β in human urine. J. Endocr. **16**, 49 (1957).
— KELLAR, R., MATTHEW, G.D.: Preliminary observations on urinary oestrogen excretion in certain gynaecological disorders. J. Obstet. Gynaec. Brit. Emp. **66**, 177 (1959).
— MATTHEW, G.D.: The application of urinary measurements to problems in gynecology. Recent Progr. Hormone Res. **18**, 337 (1962).
BROWN, W.E., BRADBURY, J.T.: Trans. Amer. Soc. Stud. Steril. **3**, 117 (1947).
— — The use of the human vaginal smear in the assay of oestrogens. J. clin. Endocr. **9**, 725 (1949).
BUCHHOLZ, H.U.: Die Capillarresistenzveränderungen während des weiblichen Zyklus und ihre Beziehung zu den Ovarialhormonen. Medizinische **24**, 617 (1953).
BUCHHOLZ, R.: Untersuchungen über die Beeinflussung der Gonadotropinausscheidung beim Menschen durch Keimdrüsenhormone. Geburtsh. Frauenheilk. **19**, 851 (1959).
— NOCKE, L., NOCKE, W.: Der Einfluß von Gestagenen auf die Urinausscheidung von Hypophysengonadotropinen, Oestrogenen und Pregnandiol bei Frauen jenseits der Menopause und während des Menstrualzyklus. Int. J. Fertil. **9**, 231 (1964).
BURGER, H.: Über das Verhalten der Cholinesterase des Endometriums bei der Frau im Verlauf des Menstruationscyclus. Arch. Gynäk. **190**, 1 (1957).
BUXTON, C.L., ATKINSON, WM. B.: Hormonal factors involved in the regulation of basal body temperature during the menstrual cycle and pregnancy. J. clin. Endocr. **8**, 544 (1948).
CALLANTINE, M.R.: Non steroidal estrogen antagonists. Clin. Obstet. Gynec. **10**, 74 (1967).
CAMPOS DA PAZ, A.: Studies on cristallization of cervical mucus and its relationship to cervical receptivity of spermatozoa. Transactions Internat. and 4th Amer. Congr. Obstet. Gynec., G.W. KOSMAK, Ed. St. Louis: C.V. Mosby Co. 1954.
CANTAROW, A., RAKOFF, A.E., PASCHKIS, K.E., HANSEN, L.P., WALKING, A.A.: Excretion of exogenous and endogenous estrogen in bile of dogs and humans. Proc. Soc. exp. Biol. (N.Y.) **52**, 256 (1943).
CAREY, H.M.: Progesterone. In: Modern trends in human reproductive physiology, vol. 1, p. 92. London: Butterworths 1963.
CLAUBERG, C.: Zur Physiologie und Pathologie der Sexualhormone, im besonderen des Hormons des Corpus luteum. 1. Mitteilung. Der biologische Test für das Luteohormon (das spezifische Hormon des Corpus luteum) am infantilen Kaninchen. Zbl. Gynäk. **54**, 2757 (1930).
CLIFT, A.F.: Observations on certain rheological properties of human cervical secretion. Proc. roy. Soc. Med. **39**, 1 (1945).
COHEN, M.R., STEIN, J.F., KAYE, B.M.: Spinnbarkeit: A characteristic of cervical mucus. Fertil. Steril. **3**, 20 (1952).
COLLETT, M.E., WERTENBERGER, G.E., FISKE, V.M.: The effect of age upon the pattern of the menstrual cycle. Fertil. Steril. **5**, 437 (1954).
COOPERMAN, N.R.: The relationship of adolescent menstruation to body temperature and sterility. Amer. J. Obstet. Gynec. **57**, 701 (1949).
CORNER, G.W., ALLEN, W.M.: Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of corpus luteum. Amer. J. Physiol. **88**, 326 (1929).
CSAPO, A.: The mechanism of effect of the ovarian steroids. Recent Progr. Hormone Res. **12**, 405 (1956).
— The role of progesterone in the maintenance and termination of pregnancy. In: Brook Lodge Symposium on Progesterone, BARNES, A.C., Ed. Augusta, Mich.: Brook Lodge Press 1961.
— Model experiments and clinical trials in the control of pregnancy and parturition. Amer. J. Obstet. Gynec. **85**, 359 (1963).
CSAPO, A.I., PINTO-DANTAS, C.R.: The cyclic activity of the non-pregnant human uterus. Fertil. Steril. **17**, 34 (1966).
DALGAARD, J.B.: The blood vessels of the human endometrium. Acta obstet. gynec. scand. **26**, 342 (1946).
DANFORTH, D.N., BAYER, P.K., GRAFF, S.: Fluctuations in weight, haematocrit and plasma protein with the menstrual cycle. Endocrinology **39**, 188 (1946).
DICZFALUSY, E., LAURITZEN, CH.: Oestrogene beim Menschen. Berlin-Göttingen-Heidelberg: Springer 1961.
DÖRING, G.K.: Über die relative Sterilität in den Jahren nach der Menarche. Geburtsh. Frauenheilk. **23**, 30 (1963).
— Über die relative Häufigkeit des anovulatorischen Zyklus im Leben der Frau. Arch. Gynäk. **199**, 115 (1963).
— Zahlen zur Physiologie des Zyklus. Fortschr. Med. **84**, 694 (1966).
— WEBER, G.: Über Schwankungen des Eiweißgehalts des Blutserums im Rhythmus des Menstruationszyklus mit Hilfe der Bestimmungen des spezifischen Gewichts des Serums. Arch. Gynäk. **179**, 442 (1951).
DOYLE, J.B., EWERS, F.D.: The fertility tester. J. Amer. med. Ass. **170**, 45 (1959).
ECKSTEIN, P.: Ovarian physiology in the non-pregnant female. In: The ovary, vol. I, p. 311, S. ZUCKERMAN, Ed. New York and London: Academic Press 1962.
EDGREN, R.A., JONES, R.C., PETERSON, D.L.: A biological classification of progestational agents. Fertil. Steril. **18**, 239 (1967).
EFFKEMANN, G.: Die Pseudomenstruation als Sterilitätsursache bei der erwachsenen Frau. Zbl. Gynäk. **63**, 153 (1939).
ELERT, R.: Über den Mechanismus der thermogenetischen Wirkung des Progesterons. Geburtsh. Frauenheilk. **11**, 325 (1951).
ENGBRING, N.H., ENGSTROM, W.W.: Effects of estrogen and testosterone on circulating thyroid hormone. J. clin. Endocr. **19**, 783 (1957).
ENGLE, E.T., SHELESNYAK, M.C.: First menstruation and subsequent menstrual cycles of prepubertal girls. Hum. Biol. **6**, 431 (1934).
ENGSTROM, W.W., MARKHARDT, B., LIEBMAN, A.: Effect of estrogen on serum precipitable iodine. Proc. Soc. exp. Biol. (N.Y.) **81**, 582 (1952).
FELDING, S.: A study of anovulatory menstruation based on 561 endometrial biopsies. Acta obstet. gynec. scand. **28**, 78 (1949).
FERIN, J.: Relative duration of action of natural and synthetic estrogens administered parenterally in woman with estrogen defiency. J. clin. Endocrin. **12**, 28 (1952).
FLUHMANN, C.F.: The length of the human menstrual cycle. Amer. J. Obstet. Gynec. **27**, 73 (1934).
— Irregularity of the stages of the menstrual cycle. Western J. Surg. **65**, 265 (1957).
FOLLEY, S.Y., MALPRESS, F.H.: Hormonal control of mammary growth. In: The hormones, PINCUS-THIMANN, Ed., vol. I, p. 695, New York: Acad. Press 1948.
FOTHERBY, K.: The ovarian production of a pregnanetriol precursor. J. Endocr. **25**, 19 (1962).

FRAENKEL, L.: Das zeitliche Verhalten von Ovulation und Menstruation. Zbl. Gynäk. **35**, 1591 (1911).
FRIEDBERG, V.: Über die Oedementstehung in der Schwangerschaft. Geburtsh. Frauenheilk. **19**, 563 (1959).
FUHRMANN, K.: Untersuchungen über die fibrinolytische Aktivität des Endometriums. Zbl. Gynäk. **84**, 1457 (1962).
GIESEN, W.: Die Wirkung eines subkutan implantierten Oestradiolkristalles bei der kastrierten Frau. Geburtsh. Frauenheilk. **5**, 219 (1943).
GÖLTNER, E., GAILER, H. J.: Blutverlust bei der Menstruation. Zbl. Gynäk. **86**, 1177 (1964).
GOLDZIEHER, M. A.: Treatment of excessive growth in the adolescent female. J. clin. Endocr. **16**, 249 (1956).
GRANT, G. A., BEALL, D.: Studies on estrogen conjugates. Recent Progr. Hormone Res. **5**, 307 (1950).
GREENBLATT, R. B.: A new clinical test for the efficacy of progesterone compounds. Amer. J. Obstet. Gynec. **76**, 626 (1958).
— BROWN, N. H.: The storage of estrogen in human fat after estrogen administration. Amer. J. Obstet. Gynec. **63**, 1361 (1952).
GUNN, D. L., JENKIN, P. M., GUNN, A. L.: Menstrual periodicity, statistical observations on a large sample of normal cases. J. Obstet. Gynaec, Brit. Emp. **44**, 839 (1937).
HAMAN, J. O.: The length of the menstrual cycle. Amer. J. Obstet. Gynec. **43**, 870 (1942).
HARTMAN, C. G.: Science and the safe period. Baltimore: Williams & Wilkins 1962.
HAUSER, G. A.: Die Rolle des neurovegetativen Nervensystems in der Geburtshilfe und Gynaekologie. Fortschr. Geburtsh. Gynäk. **10**, 160 (1960).
HELLER, C. G., CHANDLER, R. E., MEYERS, G. B.: Effect of small and large doses of diethylstilbestrol (Estrogen) upon menopausal symptoms, vaginal smear and urinary gonadotropins in 23 oophorectomized women. J. clin. Endocr. **4**, 109 (1944).
HERVEY, G. W., MCINTIRE, R. T., WATSON, V.: Low hemoglobin levels in women as revealed by blood donor records. J. Amer. med. Ass. **149**, 1127 (1952).
HESS, W. R.: Funktionelle Organisation des vegetativen Nervensystems. Basel: Benno Schwabe 1948.
HITSCHMANN, F., ADLER, L.: Der Bau der Uterusschleimhaut des geschlechtsreifen Weibes mit besonderer Berücksichtigung der Menstruation. Monatsschr. Geburtsh. Gynäk. **27**, 1 (1908).
HOFF, F.: Vegetatives Nervensystem und innere Sekretion. In: Lehrbuch der speziellen, pathologischen Physiologie (5. Aufl.). Jena: Fischer 1944.
HOFFMANN, I., OBER, K. G., SCHMITT, A.: Beobachtungen an einer Scheidenendometriose. Geburtsh. Frauenheilk. **13**, 881 (1953).
HOHLWEG, W.: Die Hormone der Keimdrüsen. In: Biologie und Pathologie des Weibes, SEITT-AMREICH, Ed. Wien: Urban & Schwarzenberg 1952.
HOOKER, C. W., FORBES, T. R.: A bio-assay for minute amounts of progesterone. Endocrinology **41**, 158 (1947).
HUHNER, M.: Sterility in the male and female and its treatment. New York: Rebman 1913.
HYTTEN, F. E., CHEYNE, G. A., KLOPPER, A. J.: Iron loss at menstruation. J. Obstet. Gynaec. Brit. Cwlth **71**, 255 (1964).
INHOFFEN, H. H., HOHLWEG, W.: Neue per os wirksame weibliche Keimdrüsenhormonderivate: 17-Aethinyl-oestradiol und Pregnen-in-on-3-ol-17. Naturwissenschaften **26**, 96 (1938).
ISRAEL, S. L., SCHNELLER, O.: The thermogenetic property of progesterone. Fertil. Steril. **1**, 53 (1950).
ITTRICH, G.: Eine neue Methode zur chemischen Bestimmung der oestrogenen Hormone im Harn. Hoppe-Seylers Z. physiol. Chem. **312**, 1 (1958).
— Untersuchungen über die Extraktion des roten Kober-Farbstoffs durch organische Lösungsmittel zur Oestrogenbestimmung im Harn. Acta endocr. (Kbh.) **35**, 34 (1960).
— Eine Methode für die klinische Routinebestimmung der Harnoestrogene. Zbl. Gynäk. **82**, 429 (1960).
JOHANNISON, E., GEMZELL, C. A., DICZFALUSY, E.: Effects of a single injection of human pituitary follicle-stimulating hormone on urinary oestrogens and the vaginal smear in amenorrhoeic women. J. clin. Endocr. **21**, 1068 (1961).
JOWSEY, J.: Microradiography of bone resorption. In: Mechanism of hard tissue destruction, SOGNNAES, R. J., Ed. Publ. No 75, Amer. Ass. for the Advancement of Science, p. 447. Washington, D. C. 1963.
JUNKMANN, K.: Die Androgenbildung im Ovar. In: Moderne Entwicklungen auf dem Gestagengebiet, H. NOWAKOWSKI, Ed. Berlin-Göttingen-Heidelberg: Springer 1960.
KAISER, I. H.: Newer concepts of menstruation. Amer. J. Obstet. Gynec. **56**, 1037 (1948).
KAUFMANN, C.: Umwandlung der Uterusschleimhaut einer kastrierten Frau aus dem atrophischen Stadium in das der sekretorischen Funktion durch Ovarialhormone. Zbl. Gynäk. **56**, 2058 (1932).
— MÜHLBOCK, O.: Ovarialfunktion und Lipoidstoffwechsel. I. Die Beziehungen zwischen Cholesterinstoffwechsel und Ovarialfunktion. Arch. Gynäk. **134**, 603 (1928).
— — Ovarialfunktion und Lipoidhaushalt. III. 1. Über die Verteilung des Blutcholesterins in freies und verestertes Cholesterin in den verschiedenen Funktionsphasen der Keimdrüse. 2. Der Gesamtfettsäuregehalt des Blutes bei regelmäßiger, bei unregelmäßiger und fehlender Ovarialfunktion. Arch. Gynäk. **139**, 254 (1930).
— ZANDER, J.: Moderne Entwicklungen auf dem Gebiet der weiblichen Sexualhormone. Ciba Symposium **7**, 146 (1959).
KING, J. L.: Menstrual intervals. Amer. J. Obstet. Gynec. **25**, 583 (1933).
KISTNER, R. W.: Therapeutic application of progestational compounds in gynecology. In: Advances in Obstet. Gynec., vol. I, p. 391, MARCUS, ST. L., C. C. MARCUS, Eds. Baltimore: Williams & Wilkins 1967.
KLOPPER, A. I.: The excretion of pregnanediol during the normal mentrual cycle. J. Obstet. Gynaec. Brit. Emp. **64**, 504 (1957).
KLOPPER, A. I., MITCHIE, E. A., BROWN, J. B.: A method for the determination of urinary pregnanediol. J. Endocr. **12**, 209 (1955).
— — The excretion of urinary pregnanediol after the administration of progesterone. J. Endocr. **13**, 360 (1956).
— STRONG, J. A., COOK, L. R.: The excretion of pregnanediol and adrenocortical activity. J. Endocr. **15**, 180 (1957).
KNAUS, H.: Über den Zeitpunkt der Konzeptionsfähigkeit des Weibes im Intermenstruum. Münch. med. Wschr. **76**, 1157 (1929).
— Die Physiologie der Zeugung des Menschen, 4th ed. Vienna: Maudrich 1953.
KOSS, L. G.: Diagnostic cytology and its histopathological bases. Philadelphia: J. B. Lippincott 1961.
KUPPERMAN, H. S., EPSTEIN, J. A., MEYER, M. D., BLATT, H. G., STONE, A.: The diagnostic and therapeutic use of intravenous estrogen in anovulation. Fertil. Steril. **9**, 26 (1958).
LAIDLAW, J. C., RUSE, J. L., GORNALL, A.: The influence of oestrogen and progesterone on aldosterone excretion. J. clin. Endocr. **22**, 161 (1962).
LAJOS, L., ILLEI, G., KECSKÉS, L., GÖRCS, J., MUTSCHER, F., KOBOR, J.: Hyperoestrogenism after the menopause. J. Obstet. Gynaec. Brit. Cwlth **70**, 1016 (1963).
LANDAU, R. L., BERGENSTAL, D. M., LUGIBIHL, K., KASCHT, M. E.: The metabolic effects of progesterone in man. J. clin. Endocr. **15**, 1194 (1955).
— LUGIBIHL, K., DIMICK: Metabolic effects in man of steroids with progestational activity. Ann. N.Y. Acad. Sci. **71**, 588 (1958).
LATZ, L. J., REINER, E.: Natural conception control. J. Amer. med. Ass. **105**, 1241 (1935).
LAURITZEN, CH.: In: Oestrogene beim Menschen, DICZFALUSY, E., C. LAURITZEN, Eds. Berlin-Göttingen-Heidelberg: Springer 1961.

LAURITZEN, CH.: Das Klimakterium. Med. Mitt. (Schering) **29**, 2 (1968).
LLOYD, C.W., LOBOTSKY, J., SEGRE, E.J., KOBAYASKI, T., TAYMOR, M.L., BATT, R.E.: Plasma testosterone and urinary 17-Ketosteroids in women with hirsutism and polycystic ovaries. J. clin. Endocr. **26**, 314 (1966).
LOESER, A.A.: Action of intravenously injected sex hormones and other substances on blood flow in human endometrium. J. Obstet. Gynaec. Brit. Emp. **55**, 17 (1948).
LORAINE, J.A.: The clinical application of hormone assay, p. 164, 174, 222. Edinburgh and London: Livingstone 1958.
MAELT, D.I., LUBIN, D.S.: A phyto-pharmacological study of menstrual toxin. J. Pharmocol. exp. Ther. **22**, 413 (1924).
MARKEE, J.E.: Menstruation in intraocular endometrial transplants in the rhesus monkey. Contr. Embryol. Carneg. Instn **28**, 219 (1940).
— The morphological and endocrine basis for menstrual bleeding. In: Progress in gynecology, vol. II, J. v. MEIGS, S.H. STURGIS, Eds. New York: Grune & Stratton 1950.
MARTINEZ-MANAUTOU, J.: Antiovulatory activy of several synthetic and natural estrogens. In: Ovulation, GREENBLATT, R.B., Ed. Philadelphia: Lippincott 1966.
MATSUMOTO, S., NOGAMI, Y., OHKURI, S.: Statistical studies on menstruation. A criticism on the definition of normal menstruation. Gunma J. med. Sci. **11**, 294 (1962).
MCSWEENEY, D.J., SBARRA, A.J.: A new cervical mucus test for hormonal appraisal. Amer. J. Obstet. Gynec. **88**, 705 (1964).
MIKHAIL, G.: Sex steroids in blood. Clin. Obstet. Gynec. **10**, 29 (1967).
MOLEN, H.J. VAN DER, GROEN, D.: Determination of progesterone in human peripheral blood using gas-liquid chromatography with electron capture detection. J. clin. Endocr. **25**, 1625 (1965).
NAPP, J.H.: Habilitationsschrift. Hamburg, 1954.
NEEF, J.C. DE: Clinical endocrine cytology. New York: Hoeber 1967.
NEILL, J.D., JOHANSSON, E.D.B., DATTA, J.K., KNOBIL, E.: Relationship between the plasma levels of luteinizing hormone and progesterone during the normal menstrual cycle. J. clin. Endocr. **27**, 1167 (1967).
NEVINNY-STICKEL, J.: Inhibition of ovulation determined by estimation of pregnanediol excretion. Int. J. Fertil. **9**, 57 (1964).
NOYES, R.W., HERTIG, A.T., ROCK, J.: Dating of endometrial biopsy. Fertil. Steril. **1**, 1 (1950).
OBER, K.G.: Das Ovar. In: LABHART, A., Klinik der inneren Sekretion, 1st ed. Berlin-Göttingen-Heidelberg: Springer 1957.
— KLEIN, I., WEBER, M.: Zur Frage einer Progesteronbehandlung. Arch. Gynäk. **184**, 543 (1954).
— WEBER, M.: Beitrag zur Progesteronwirkung. Klin. Wschr. **3**, 53 (1951).
OBER, W.B., KAISER, G.A.: Pregnanediol: Methods, limitations and applications. Amer. J. clin. Path. **35**, 297 (1961).
OLIVER, M.F., BOYD, G.S.: Changes in the plasma lipids during the menstrual cycle. Clin. Sci. **12**, 217 (1953).
OVERSTREET, E.W.: An evaluation of infertility factors. Calif. Med. **69**, 1 (1948).
PALMER, R.: La détermination hormonal de l'apparition et de la disparition du glaires filantes du col utérin. Soc. Biol. **22**, 366 (1941).
— MARCILLE, S.: Le mucus cervical normal et pathologique. Gynéc. Obstét. **41**, 455 (1941).
PAPANICOLAOU, G.N.: The sexual cycle in the human female as revealed by vaginal smears. Amer. J. Anat. **52**, 519 (1933).
— A new procedure for staining vaginal smears. Science **95**, 438 (1942).
— General survey of a vaginal smear and its use in research and diagnosis. Amer. J. Obstet. Gynec. **51**, 316 (1946).
PEARLMAN, W.H.: Circulating steroid hormone levels in relation to steroid hormone production. Ciba Found. Coll. Endocr. **1**, 233 (1957).
PHELPS, D.: Menstruation. In: VELARDO, J.T. (Ed.), Essentials of human reproduction. New York: Oxford University Press 1958.
PHILLIPS, L.L., BUTTLER, B.C., TAYLOR, H.C.: A study of cytofibrinokinase and fibrinolysin in extracts of tissue from human myometrium, endometrium, decidua and placenta. Amer. J. Obstet. Gynec. **71**, 342 (1956).
PLOTZ, E.J.: Die Anwendung radioaktiver Isotope in der Erforschung des Gestagenstoffwechsels in der Schwangerschaft. 6th Symposium der Deutschen Gesellsch. für Endokrinologie, p. 21. Berlin-Göttingen-Heidelberg: Springer 1960.
PRICE, D.C., FORSYTH, E.M., COHN, S.H., CRONKITE, E.P.: The study of menstrual and other blood loss, and consequent iron deficiency, by Fe 59 whole body counting. Canad. med. Ass. J. **90**, 51 (1964).
PSCHYREMBEL, W.: Praktische Gynäkologie, 4th ed. Berlin: W. de Gruyter 1968.
PUCK, A.: Der Einfluß des Oestriols auf das Sekret der Cervix uteri. Geburtsh. Frauenheilk. **18**, 998 (1958).
PUNDEL, J.P.: Les frottis vaginaux endocriniens. Paris: Masson 1952.
— Acquisitions récentes en cytologie vaginale hormonale. Paris: Masson 1957.
— Précis de colpocytologie hormonale. Paris: Masson 1966.
RAUSCHER, H.: Vergleichende Untersuchungen über das Verhalten des Vaginalabstrichs, der Zervixfunktion und der Basaltemperatur in zweiphasigen Zyklen. Geburtsh. Frauenheilk. **14**, 327 (1954).
REYNOLDS, S.R.M.: Studies on the uterus. VI. The effect of oestrin on the uterine fistula during pseudo-pregnancy. Amer. J. Physiol. **98**, 230 (1931).
— Physiologic basis of menstruation; summary of current concepts. J. Amer. med. Ass. **135**, 552 (1947).
RICE, B.F., HAMMERSTEIN, J., SAVARD, K.: Steroid hormone formation in the human ovary: II. Action of gonadotropins *in vitro* in the corpus luteum. J. clin. Endocr. **24**, 606 (1964).
RICE, B.F., HAMMERSTEIN, J., SAVARD, R.: Steroid hormone formation in the human ovary: III. Action of gonadotropins on testosterone synthesis by normal ovarian stromal tissue. Steroids **4**, 199 (1964).
— SAVARD, K.: Steroid hormone formation in the human ovary: IV. Ovarian stromal compartment: Formation of radioactive steroids from acetate-1-14 C and action of gonadotropins. J. clin. Endocr. **26**, 593 (1966).
RIONDEL, A., TAIT, J.F., TAIT, S.A.S., GUT, M., LITTLE, B.: Estimation of progesterone in human peripheral blood using S-thiosemicarbazide. J. clin. Endocr. **25**, 229 (1965).
ROCK, J.: Physiology of human conception. New Engl. J. Med. **240**, 804 (1949).
— MATSON, D.D.: The incidence of anovulatory menstruation among patient of low fertility. Amer. J. Obstet. Gynec. **37**, 3 (1939).
RÖTTGER, H., HECKENBACH, E.M.: Der Einfluß der Sexualhormone auf den Wasserhaushalt der Frau. Arch. Gynäk. **190**, 95 (1957).
ROLAND, M.: Progestagen therapy. Springfield (Ill.): Charles C. Thomas 1965.
ROTH, O.A.: Über die Bedeutung des Vaginalabstrichverfahrens (vaginal smear) nach Papanicolaou zur Zyklusdiagnose. Dtsch. med. Wschr. **75**, 1719 (1950).
RUNNEBAUM, B., MOLEN, H. VAN DER, ZANDER, J.: Steroids in human peripheral blood of the menstrual cycle. Steroids, Suppl. **2**, 189 (1965).
RYAN, K.J.: Synthesis of hormones in the ovary. In: The ovary, GRADY, H.G., SMITH, D.E., Eds., p. 69. Baltimore: Williams & Wilkins 1963.
— Steroid metabolism in the human ovary. In: Advances in obstet. gynec., vol. I, MARCUS, ST.L., C.C. MARCUS, Eds. Baltimore: Williams & Wilkins 1967.

— PETRO, Z.: Steroid biosynthesis by human ovarian granulosa and theca cells. J. clin. Endocr. **26**, 46 (1966).
— SMITH, O.W.: Biogenesis of steroid hormones in the human ovary. Recent Progr. Hormone Res. **21**, 367 (1965).
RYBO, G.: Clinical and experimental studies on menstrual blood loss. Menstrual blood loss in relation to parity and menstrual pattern. Acta obstet. gynec. scand. **45**, Suppl. 7 (1966).
RYDBERG, E.: Observations on the crystallization of the cervical mucus. Acta obstet. gynec. scand. **28**, 172 (1948).
SABINE, M.: Le mucus cervical normal et pathologique. Paris: Lavergne 1941.
SANDBERG, A.A., SLAUNWHITE, W.R., JR.: Studies on phenolic steroids in human subjects. II. The metabolic fate and hepato-biliari-enteric circulation of C_{14}-Estrone and C_{14}-estradiol in women. J. clin. Invest. **36**, 1266 (1957).
SAVARD, K., MARSH, J.M., RICE, B.F.: Gonadotropins and ovarian steroido-genesis. Recent Progr. Hormone Res. **21**, 285 (1965).
SCHLEGEL, J.U.: Arteriovenous anastomoses in the endometrium in man. Acta anat. (Basel) **1**, 284 (1945/46).
SCHMIDT-MATTHIESEN, H.: Das normale menschliche Endometrium. Stuttgart: Thieme 1963.
SCHMITT, A.: Eine Gradeinteilung für die funktionelle Zytodiagnostik in der Gynäkologie. Geburtsh. Frauenheilk. **13**, 593 (1953).
SCHREINER, W.E., VILLEE, C.A.: Oxidative Phosphorylation in the human placenta. Amer. J. Obstet. Gynec. **91**, 961 (1965).
SCHRÖDER, R.: Der mensuelle Genitalcyclus des Weibes und seine Störungen. In: VEIT-STOECKEL, Handbuch der Gynaekologie, Bd. 1/2, p. 234. Munich: J.F. Bergmann 1928.
SHORR, E.: New technic for staining vaginal smears. Science **91**, 321 (1940).
SHORT, R.U.: Ovarian steroid synthesis and secretion *in vivo*. Recent Progr. Hormone Res. **20**, 303 (1964).
SIEGLER, S., SIEGLER, A.M.: Evaluation of the basal body temperature. An analysis of 1012 basal body temperature recordings. Fertil. Steril. **2**, 287 (1951).
SIMS, J.M.: Illustrations of the value of the microscope in the treatment of the sterile condition. Brit. med. J. **1868 II**, 465.
SMITH, O.W.: "Menstrual toxin". In: Menstruation and its disorders, ENGLE, E.T., Ed. Springfield (Ill.): Thomas 1950.
SMOLKA, H., SOOST, H.J.: Grundriß und Atlas der gynäkologischen Zytodiagnostik. Stuttgart: G. Thieme 1965.
STOLL, P., JAEGER, J., DALLENBACH-HELLWEG, G.: Gynäkologische Cytologie. Berlin-Heidelberg-New York: Springer 1968.
SULIMOVICI, S., LUNENFELD, B., SHELESNYAK, M.D.: Practical method for estimation of urinary pregnanediol and allopregnanediol using thin layer chromatography. Acta endocr. (Kbh.) **49**, 57 (1965).
SVENDSEN, R.: A double isotopic derivative method for the quantitative determination of oestrone and 17β-oestradiol in plasma. Acta endocr. (Kbh.) **35**, 161 (1960).
— SÖRENSEN, B.: The plasma concentration of unconjugated estrone and 17β-oestradiol during the normal menstrual cycle. Acta endocr. (Kbh.) **47**, 245 (1964).
SWYER, G.I.M., LITTLE, V.: Clinical assessment of orally active progestagens. Proc. roy. Soc. Med. **55**, 861 (1962).
SZONTAGH, F.E.: Therapeutic and contraceptive actions and uses of oral progestagens. In: Symposium on Reprod., K. LISSAK, Ed. Budapest: Akademiai Kiado 1967.
THORN, G.W., NELSON, K.R., THORN, D.W.: A study of the mechanism of edema associated with menstruation. Endocrinology **22**, 155 (1938).
TIETZE, K.: Der weibliche Zyklus und seine Störungen. In: Biologie und Pathologie des Weibes, 2nd ed. Bd. II, SEITZ, L., AMREICH, A.I., Eds. Munich: Urban & Schwarzenberg 1952.
TURNER, D.A., JONES, G.E.S., SARLOS, I.J., BARNES, A.C., COHEN, R.: Determination of urinary prenanediol by gas chromatography. Analyt. Biochem. **5**, 99 (1963).
UFER, J.: Hormontherapie in der Frauenheilkunde, 3rd ed. Berlin: W. de Gruyter 1966.
VASQUEZ ROCHA, M., CLERICI, M.E., TRAVELLA DE GIORDANO, I.: La function menstruel desde el punto de vista estadistico. Día méd. **15**, 669 (1943).
VIERGIVER, E., POMMERENKE, W.T.: Measurements of cyclic variations in quantity of cervical mucus: Correlation with basal temperature. Amer. J. Obstet. Gynec. **48**, 321 (1944).
— — Viscosity of cervical mucus and its correlation with basal temperature. Amer. J. Obstet. Gynec. **51**, 192 (1946).
VILLEE, CL. A.: The influence of oestrogens on uterine and placental enzymes. In: Modern trends in human reproductive physiology, vol. 1, CAREY, H.M., Ed. London: Butterworths 1963.
VOLLMANN, R.: Variationsstatistische Analyse der Phasen des Genitalzyklus der Frau durch Auswertung des Intermenstrualschmerzes als Indikator für den Ovulationstermin. Mschr. Geburtsh. Gynäk. **110**, 115, 193 (1940).
— Die Länge des Prämenstruum in Regression zum Alter der Frau. Gynaecologia (Basel) **135**, 78 (1953).
— The degree of variability of the length of the menstrual cycle in correlation with age of women. Gynaecologia (Basel) **142**, 310 (1956).
VOLLMANN-SIEHR: Untersuchungen über die Körpertemperatur der Frau in Korrelation zu den Phasen ihres Genitalzyklus. Diss. Zürich, 1940. Mschr. Geburtsh. Gynäk. **111**, 41, 121 (1940).
VORYS, N., ULLERY, J.C., STEVENS, V.: The effects of sex steroids on gonadotropins. Amer. J. Obstet. Gynec. **93**, 641 (1965).
WEISS, G., BELLER, F.K.: Fibrinolytic activity in the human uterus. Obstet. Gynec. **31**, 581 (1968).
WERTH, G.: Die gonadotropen Hormone (1). Die gonadotropen Hormone (2). Arzneimittel-Forsch. **5**, 409, 735 (1955).
WIED, G.L.: Technische und terminologische Hinweise zur zytologischen Hormondiagnostik aus dem Vaginalabstrich. Röntgen- und Lab.-Prax. **6**, 209 (1953).
— Oestradiol-valerianat und Oestradiol-undecylenat: zwei neue protrahiert wirkende Oestrogene. Wirkungsvergleich mit Oestradiol-benzoat. Geburtsh. Frauenheilk. **14**, 45 (1954).
WIED, G.L.: Oestradiol-valerianat und Oestradiol-undecylenat: zwei neue protrahiert wirkende Oestrogene. Wirkungsvergleich mit Oestradiol-benzoat. Geburtsh. Frauenheilk. **14**, 45 (1954).
WOOLEVER, C.A.: Daily plasma progesterone levels during menstrual cycle. Amer. J. Obstet. Gynec. **85**, 981 (1963).
ZINSER, H.K.: Die Zytologie in der Gynäkologie. Jena: Fischer 1957.
ZANDER, J.: Progesterone in human blood and tissues. Nature (Lond.) **174**, 406 (1954).
— Steroids in the human ovary. J. biol. Chem. **232**, 117 (1958).
— Gestagene im menschlichen Organismus. Bull. Soc. roy. belg. Gynéc. Obstét. **28**, 423 (1958).
— Neuere Erkenntnisse über die natürlichen Gestagene im menschlichen Organismus. In: Moderne Entwicklungen auf dem Gestagengebiet, H. NOWAKOWSKI, Ed. Berlin-Göttingen-Heidelberg: Springer 1960.
— Les androgènes ovariens. Rev. franç. Endocr. clin. **4**, 409 (1963).
— Der menstruelle Zyklus. In: Gynäkologie und Geburtshilfe, KÄSER, O., Ed., Bd. I, p. 250. Stuttgart: Thieme 1969.
— HOLZMANN, K.: Störungen des menstruellen Zyklus und ihre Behandlung. In: Gynäkologie und Geburtshilfe. KÄSER, O., Ed., Bd. I., p. 315. Stuttgart: Thieme 1969.
— BORBES, T.R., MÜNSTERMANN, A.M. v., NEHER, R.J.: Δ^4-3-Ketopregnene-20α-ol and Δ^4-3-Ketopregnene-20β-ol, two natural occurring metabolites of progesterone. J. clin. Endocr. **18**, 337 (1958).

ZANDER, J., BRENDLE, E., MÜNSTERMANN, A.M.v., DICZFALUSY, E., MARTINSEN, B., TILLINGER, G.K.: Identification and estimation of oestradiol-17 and oestrone in human ovaries. Acta obstet. gynec. scand. **38**, 724 (1959).
— RUNNEBAUM, B.: Progesterone in human blood. In: Methods in hormone research, DORFMANN, R., Ed. New York: Academic Press 1967.
ZONDEK, B., ROZIN, S.: Cervical mucus arborization: its use in determining of corpus luteum function. Obstet. Gynec. **3**, 463 (1954).

Regulation of Ovarian Functions

AKERT, K.: Das limbische System und seine funktionelle Bedeutung. In: Relaxation und Sedation des menschlichen Uterus, NEUWEILER, W., M. BERGER, Eds. Fortschr. Geburtsh. Gynäk. **19**, 4 (1964).
ARIMURA, A., SCHALLY, A.V., BOWERS, C.Y.: Corticotropin releasing activity of lysine vasopressin analogues. Endocrinology **84**, 579 (1969).
— — SAITO, T., MÜLLER, E.E., BOWERS, C.Y.: Induction of ovulation in rats by highly purified pig LH-releasing factor (LRF). Endocrinology **80**, 515 (1967).
BARGMANN, W.: Das Zwischenhirn-Hypophysensystem. Berlin-Göttingen-Heidelberg: Springer 1954.
BARRACLOUGH, C.A.: Influence of age on the response of preweaning female mice to testosterone propionate. Amer. J. Anat. **97**, 493 (1955).
— Production of anovulatory sterile rats by single injections of testosterone propionate. Endocrinology **68**, 62 (1961).
— LEATHEM, J.H.: Infertility induced in mice by a single injection of testosterone propionate. Proc. Soc. exp. Biol. (N.Y.) **85**, 673 (1954).
DAVID, M.A., FRASCHINI, F., MARTINI, L.: Parallélisme entre le contenu hypophysaire en FSH et le contenu hypothalamique en FSH-RF (FSH-Releasing factor). C.R. Acad. Sci. (Paris) **261**, 2249 (1965).
— — — An *in-vivo* method for evaluating the hypothalamic follicle stimulating hormone releasing factor. Experientia (Basel) **21**, 483 (1965).
— — — Control of LH secretion. Role of a "short" feedback mechanism. Endocrinology **78**, 55 (1966).
DIEPEN, R.: Der Hypothalamus. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. IV/7, W. VON MÖLLENDORF, W. BARGMANN, Eds. Berlin-Göttingen-Heidelberg: Springer 1962.
EVERETT, J.W.: Functional corpora lutea maintained for months by autografts of rat hypophyses. Endocrinology **58**, 786 (1956).
FINK, G., NALLAR, R., WORTHINGTON, W.C.: The demonstration of luteinizing hormone releasing factor in hypophysial portal blood of pro-oestrus and hypophysectomized rats. J. Physiol. (Lond.) **191**, 407 (1967).
— — WORTHINGTON, W.C., JR.: Determinations of luteinizing hormone releasing factor (LRF) in hypophyseal portal blood. J. Physiol. (Lond.) **183**, 200 (1966).
FLERKO, B.: Brain mechanism controlling gonadotropin secretion and their sexual differentiation. In: Symposium on reproduction, K. LISSAK, Ed., p. 11. Budapest: Akadémiai Kiado 1967.
— SZENTAGOTHAI, J.: Oestrogen sensitive nervous structures in the hypothalmus. Acta endocr. (Kbh.) **26**, 121 (1957).
GITTES, F., KASTIN, A.J.: Effects of increasing numbers of pituitary transplants in hypophysectomized rats. Endocrine Soc. 47th Meeting Abst. 31, 1965.
GUILLEMIN, R.: Hypothalmic control of anterior pituitary study with tissue culture techniques. Fed. Proc. **14**, 211 (1955).
— Hypothalamic factors releasing pituitary hormones. Recent Progr. Hormone Res. **20**, 89 (1964).
HARRIS, G.W.: Central control of the pituitary gland. London: Arnold 1958.
— The pituitary stalk and ovulation. In: Control of ovulation, VILLEE, C.A., Ed. New York: Pergamon Press 1961.
— The central nervous system and the endocrine glands. Triangel (De.) **6**, 242 (1964).
— JACOBSOHN, D.: Functional grafts of the anterior pituitary gland. Proc. roy. Soc. B **139**, 263 (1952).
JOHNSEN, S.G.: A clinical routine method for the determination of gonadotropins in 24-hour urine samples. II. Normal values for men and women at all age groups from pre-puberty to senescence. Acta endocr. (Kbh.) **31**, 209 (1959).
JONES, I.C., BALL, J.: Ovarian-pituitary relationships. In: The ovary, vol. I, S. ZUCKERMAN, Ed. New York and London: Academic Press 1962.
JUTISZ, M.: Données récentes sur les facteurs hypothalamiques LRF et FRF. In: Fonctions endocriniennes de l'ovaire, M.F. JAYLE, Ed. Paris: Gauthier-Villars 1966.
KELLER, P.J., NAVILLE, A.H., WYSS, H.I.: Experimentelle Untersuchungen über die Wirkungsweise von Clomiphen. Gynaecologia (Basel) **165**, 192 (1968).
MARTINI, L.: Neural control of anterior pituitary functions. Recent Progr. Hormone Res. **24**, 439 (1968).
MCCANN, S.M.: A hypothalamic luteinizing-hormone-releasing factor. Amer. J. Physiol. **202**, 395 (1962).
— TALEISNIK, S., FRIEDMAN, H.M.: LH-releasing activity in hypothalamic extracts. Proc. Soc. exp. Biol. (N.Y.) **104**, 432 (1960).
MEITES, J.: The central nervous system and the secretion and release of prolactin. In: Advances in neuroendocrinology, A.V. NALBANDOV, Ed., p. 238. Urbana (Ill.): University of Illinois Press 1963.
MITTLER, J.C., MEITES, J.: *In vitro* stimulation of pituitary follicle-stimulating-hormone release by hypothalamic extracts. Proc. Soc. exp. Biol. (N.Y.) **117**, 309 (1964).
PASCHKIS, K.E., RAKOFF, A.E.: Clinical endocrinology. In: The hormones, PINCUS, G., K.V. THIMANN, Eds., vol. 3, p. 821. New York: Academic Press 1955.
POPA, G.T., FIELDING, U.: A portal circulation from the pituitary to the hypothalamic region. J. Anat. (Lond.) **65**, 88 (1930).
ROTHCHILD, J.: The central nervous system and disorders of ovulation in women. Amer. J. Obstet. Gynec. **98**, 719 (1967).
SCHALLY, A.V., BOWERS, C.Y.: *In-vitro* and *in-vivo* stimulation of the release of luteinizing hormone. Endocrinology **75**, 312 (1964).
— KASTIN, A.J.: Die hypothalamischen Hormone, welche die Freisetzung der Hypophysenhormone stimulieren oder hemmen. Triangel (De.) **9**, 19 (1969).
— KUROSHIMA, A., ISHIDA, Y.: The presence of prolactin inhibiting factor (PIF) in extracts of beef, sheep and pig hypothalami. Proc. Soc. exp. Biol. (N.Y.) **118**, 350 (1965).
— MÜLLER, E.E., AKIMURA, A., BOWERS, C.Y., SAITO, T., REDDING, T.W., SAWANO, S., PIZZOLATO, P.: Releasing factors in human hypothalamic and neurohypophysial extracts. J. clin. Endocr. **27**, 755 (1967).
— SAITO, T., AKIMURA, A., MÜLLER, E.E., BOWERS, C.Y., WHITE, W.F.: Purification of follicle-stimulating hormone-releasing factor (FSH-RF) from bovine hypothalamus. Endocrinology **79**, 1087 (1966).
SZONTAGH, F.E., UHLARIK, S.: Possibility of a direct "internal" feedback in the control of pituitary gonadotropin secretion. J. Endocr. **29**, 203 (1964).
TALWAKER, P.K., RATNER, A., MEITES, J.: *In-vitro* inhibition of pituitary polactin synthesis and release by hypothalamic extract. Amer. J. Physiol. **205**, 213 (1963).
WESTMAN, A.: Die Physiologie des Hypophysen-Hypothalamus-Systems unter besonderer Berücksichtigung der Regulation der Sexualfunktion. In: Zentrale Steuerung der Sexualfunktionen, H. NOWAKOWSKI, Eds. Berlin-Göttingen-Heidelberg: Springer 1955.
WHITE, W.F., COHEN, A.I., RIPPEL, R.H., STORY, J.C.: Some hypothalamic polyamines that deplete pituitary follicle stimulating hormone. Endocrinology **82**, 742 (1968).

The Pituitary Gonadotropins

ALBERT, A.: Procedure for routine clinical determination of urinary gonadotropin. Proc. Mayo Clin. **30**, 552 (1955).

— Separation of follicle stimulating and luteinizing hormones from urine of postmenopausal women. J. clin. Endocr. **25**, 1119 (1965).

APOSTOLAKIS, M., VOIGT, K.D.: Die Gonadotropine. Stuttgart: Thieme 1965.

BETTENDORF, G., APOSTOLAKIS, M., VOIGT, K.D.: Darstellung von Gonadotropinen aus menschlichen Hypophysen. Acta endocr. (Kbh.) **41**, 1 (1962).

— BRECKWOLDT, M.: Klinisch-experimentelle Untersuchungen mit hypophysärem Human-Gonadotropin. Arch. Gynäk. **199**, 423 (1964).

BROWN, P.S.: The assay of gonadotrophine from urine of non pregnant human subjects. J. Endocr. **13**, 59 (1955).

DONINI, P., PUZZUOLI, D., MONTEZERNOLO, R.: Purification of gonadotropin from human menopausal urine. Acta endocr. (Kbh.) **45**, 321 (1964).

FUKUSHINA, M., STEWENS, V.C., GANT, C.L., VORYS, N.: Urinary FSH and LH excretion during the normal menstrual cycle. J. clin. Endocr. **24**, 205 (1964).

GEMZELL, C.A.: Induction of ovulation in the human by human gonadotrophins. In: Progress in gynecology, vol. IV, MEIGS, J.V., S.H. STURGIS, Eds. New York and London: Grune & Stratton 1963.

— Human pituitary gonadotropins in the treatment of sterility. Fertil. Steril. **17**, 149 (1966).

— Experiences with human pituitary gonadotropins. In: Ovulation, R.B. GREENBLATT, Ed. p. 98. Philadelphia, Toronto: Lippincott 1966.

— Induction of ovulation using human pituitary or urinary FSH. Clin. Obstet. Gynec. **10**, 401 (1967).

— Ovulation. Das Medizinische Prisma (Boehringer) **1**, 3 (1969).

— DICZFALUSY, E., TILLINGER, K.G.: Clinical effect of human pituitary follicle-stimulating hormone (FSH). J. clin. endocr. **18**, 1333 (1958).

— KJESSLER, B.: Treatment of infertility after partial hypophysectomie with human pituitary gonadotropins. Lancet **1964 I**, 644.

GREEP, R.O., DYKE, H.B. VAN, CHOW, B.F.: Use of anterior lobe of prostate gland in the assay of metakentrin. Soc. exp. Biol. (N.Y.) **46**, 644 (1941).

GRÖSCHEL, U., LI, CH.H.: On the carbohydrate moiety of bovine and human pituitary gonadotropins. Biochim. biophys. Acta (Amst.) **37**, 375 (1960).

HEINRICHS, H.D., EULEFELD, F.: Untersuchungen über die Extraktion von hypophysären Gonadotropinen aus Urin. Acta endocr. (Kbh.), Suppl. **1 iii**, 1 (1960).

HERLANT, M.: Les cellules responsables de l'activité gonadotrope dans l'hypophyse humaine. In: Fonctions endocriniennes de l'ovaire, M.F. JAYLE, Ed. Paris: Gauthiers-Villars 1966.

ICHII, S., FORCHIELLI, E., DORFMAN, R.I.: *In vitro* effect of gonadotropins on the soluble cholesterol side-chain cleaving enzyme system of bovine corpus luteum. Steroids **2**, 631 (1963).

IRVINE, W.I.: Reproductive endocrinology. Edinburgh and London: Livingstone 1970.

JOHNSON, S.G.: A clinical routine method for the quantitative determination of gonadotropins in 24-hour urine samples. Acta endocr. (Kbh.) **28**, 69 (1958).

JOHNSON, S.G.: A clinical routine method for the quantitative determination of gonadotropins in 24-hour-urine samples. II. Normal values for men and women at all age groups from prepuberty to senescence. Acta endocr. (Kbh.) **31**, 209 (1959).

KELLER, P.J.: Die Bestimmung hypophysärer Gonadotropine im Urin. Gynaecologia (Basel) **156**, 3 (1963).

— The renal clearance of follicle-stimulating and luteinizing hormone in postmenopausal women. Acta endocr. (Kbh.) **53**, 225 (1966).

— Hormonal studies in endocrine disease. Germ. med. Mth. **13**, 389 (1968).

— Hypophysäre Gonadotropine: Untersuchungen über Physiologie, Pathologie und medikamentöse Beeinflussung der FSH- und LH-Sekretion bei der Frau. Habilitation, Zurich 1971.

— ROSEMBERG, E.: Estimation of pituitary gonadotropins in human plasma. J. clin. Endocr. **25**, 1050 (1965).

KISTNER, R.W.: Use of Clomiphene citrate, human chorionic gonadotropin and human menopausal gonadotropin for induction of ovulation in the human female. Fertil. Steril. **17**, 569 (1966).

KLINEFELTER, H.F., ALBRIGHT, F., GRISWOLD, G.C.: Experience with a quantitative test for normal and decreased amounts of follicle-stimulating hormone in the urine in endocrinological diagnosis. J. clin. Endocr. **3**, 529 (1943).

LUNENFELD, B., MENZI, A., VIOLET, B.: Clinical effects of human postmenopausal gonadotropin. Acta endocr. (Kbh.), Suppl. **51**, 587 (1960).

MASON, N.R., MARSH, J.N., SAVARD, K.: An action of gonadotropins *in vitro*. J. biol. Chem. **236**, 34 (1961).

MCARTHUR, J.W., INGERSOLL, F.M.: The urinary excretion of JCSH- and FSH-activity during the normal menstrual cycle. J. clin. Endocr. **18**, 1186 (1958).

MIDGLEY, A.R., JAFFE, B.R.: Regulation of human gonadotropins. IV. Correlation of serum concentration of follicle stimulating and luteinizing hormones in the menstrual cycle. J. clin. Endocr. **28**, 1699 (1968).

ODELL, W.D., SWAIN, R.W., NYDECK, M.: Molecular weight of human pituitary gonadotropins as determined by radiation inactivation of biological activity. J. clin. Endocr. **24**, 1266 (1964).

PARLOW, A.F.: A rapid biossay method for LH and factors stimulating LH secretion. Fed. Proc. **17**, 402 (1958).

— The biological half-life of LH, PM and HCG in the rat. Fed. Proc. **19**, 155 (1960).

— Bioassay of pituitary luteinizing hormone by depletion of ovarian ascorbic acid. In: Human pituitary gonadotropines, ALBERT, A., Ed., p. 300. Springfield, Ill.: C.C. Thomas 1961.

REICHERT, L.E., PARLOW, A.F.: Partial purification and separation of urinary gonadotropins of nonpregnant humans. J. clin. Endocr. **24**, 1040 (1964).

ROOS, P.: Human follicle-stimulating hormone. Dissertation, Uppsala 1968.

— GEMZELL, C.A.: The isolation of human pituitary follicle-stimulating hormone. Biochim. biophys. Acta (Amst.) **82**, 218 (1964).

ROSEMBERG, E.: Induction of ovulation with human menopausal gonadotropin. In: Ovulation, R.B. GREENBLATT, Ed., p. 118. Philadelphia, Toronto: J.B. Lippincott Comp. 1966.

— MAHER, R.E., STERN, A., DEMANY, M.: Clinical effect of gonadotropins of human origin. Case report with a two-year follow-up. J. clin. Endocr. **24**, 105 (1964).

ROSEMBERG, E.A., KELLER, P.J.: Studies on the urinary excretion of follicle-stimulating and luteinizing hormone activity during the menstrual cycle. J. clin. Endocr. **25**, 1262 (1965).

RYAN, R.J.: The luteinizing hormone content of human pituitary. I. Variations with sex and age. J. clin. Endocr. **22**, 300 (1962).

STEELMAN, S.L., POHLEY, F.M.: Assay of the follicle-stimulating hormone based on the augmentation with human chorionic gonadotropin. Endocrinology **53**, 604 (1953).

TAYMOR, M.L.: The pituitary gonadotropins. In: Progress in gynecology, vol. IV, MEIGS, J.V., S.H. STURGIS, Eds. New York and London: Grune & Stratton 1963.

WIELE, R.L. VAN DE, TURKSOY, R.N.: Treatment of amenorrhea and of anovulation with human menopausal and chorionic gonadotropins. J. clin. Endocr. **25**, 369 (1965).

Ovulation

ABARBANEL, A. R.: Transvaginal pelvioscopy. Further studies in infertility. Proc. 2nd World Congr. Fertil. and Steril. (Naples 1956) **1**, 1140 (1958).

BELLER, F. K., VOGLER, H.: Die Bestimmung des Ovulationstermines bzw. der praeovulatorischen Phase unter besonderer Berücksichtigung der physikalischen Beschaffenheit des Cervixschleims. Z. Geburtsh. Gynäk. **158**, 58 (1962).

BERGMAN, P.: Sexual cycle, time of ovulation and time of optimal fertility in women. Acta obstet. gynec. scand. **29**, Suppl. 4 (1950).

BERNOTH, Z.: Zur Zyklusanalyse aus dem Zervikalsekret. Z. Geburtsh. Gynäk. **147**, 76 (1956).

BICKENBACH, W., PAULIKOVICS, E.: Hemmung der Follikelreifung durch Progesteron. Zbl. Gynäk. **68**, 153 (1944).

BIRNBERG, C. H., KURZROK, R., LAUFER, A.: Simple test for determining ovulation time. J. Amer. med. Ass. **166**, 1174 (1958).

BRUCE, H. M., PARROT, D. M. V.: Role of olfactory sense in pregnancy block by strange males. Science **131**, 1526 (1960).

BURR, H. S., HILL, R. T., ALLEN, E.: Detection of the ovulation in the intact rat. Proc. Soc. exp. Biol. (N. Y.) **33**, 109 (1935).

CHEE, J. P., YOU, S. S.: Role of thyroid gland and estrogen in regulation of gonadotrophic activity of anterior pituitary. J. Endocr. **4**, 115 (1945).

COLTON, F. B.: 13-methyl-17-ethinyl-17-hydroxy-1,2,3,4,6,7,8-9,11,12,13,14,16,17-tetradecahydro-15H-cyclopentaphenanthren-3-one. US Patent 2, 725, 389 (1955).

DICZFALUSY, E.: Mode of action of contraceptive drugs. Amer. J. Obstet. Gynec. **100**, 136 (1968).

DONAYRÉ, J., PINCUS, G.: In: The control of fertility, PINCUS, G., Ed., p. 208, Tab. 33. New York: Academic Press 1965.

DOYLE, J. B., EWERS, F. J.: The fertility tester. J. Amer. med. Ass. **170**, 45 (1959).

ENGLE, E. T.: Reproduction in domestic animals. New York: Academic Press 1959.

ERB, H., LUDWIG, K. S.: Corpus-luteum-Bildung während Einnahme eines hormonalen Antikonzeptivums. Gynaecologia (Basel) **158**, 1 (1964).

EVERETT, J. W.: Evidence suggesting a role of the lactogenic hormone in the estrous cycle of the albino rat. Endocrinology **35**, 507 (1944).

FLOWERS, C. E., VORYS, N., STEVENS, V., MILLER, A. T., JENSEN, L.: Effects of suppression of menstruation with ethynodiol diacetate upon the pituitary, ovary, and endometrium. Amer. J. Obstet. Gynec. **96**, 784 (1966).

FLUHMANN, C. F.: Influence of thyroid on action of gonad-stimulating hormones. Amer. J. Physiol. **108**, 498 (1934).

FORD, D. H., GROSS, J.: The metabolism of I-131 labelled thyroid hormones in the hypophysis and brain of the rabbit. Endocrinology **62**, 416 (1958).

FUKUSHIMA, M., STEVENS, V. C., GANTT, C. L., VORYS, N.: Urinary FSH and LH excretion during the normal menstrual cycle. J. clin. Endocr. **24**, 205 (1964).

GARCIA, C. R.: Oral contraceptive. An appraisal and review. Amer. J. med. Sci. **253**, 718 (1967).

— Oral contraception. Clin. Obstet. Gynec. **11**, 627 (1968).

GEMZELL, C. A., ROOS, P.: Pregnancies following treatment with human gonadotropins with special reference to the problem of multiple births. Amer. J. Obstet. Gynec. **94**, 490 (1966).

GOLDZIEHER, J. W., BECERRA, C., GUAL, C., LIVINGSTON, N. B., MAGUEO, M., MOSES, L. E., TIETZE, C.: New oral contraceptive. Sequential estrogen and progestin. Amer. J. Obstet. Gynec. **90**, 404 (1964).

— LIVINGSTON, N. B., MARTINEZ-MANAUTOU, J., MOSES, L. E., RICE-WRAY, E.: Use of sequential estrogen and progestin to inhibit fertility. West. J. Surg. **71**, 187 (1963).

— MOSES, L. E., ELLIS, L. T.: Study of norethindrone in contraception. J. Amer. med. Ass. **180**, 359 (1962).

GOODLAND, R. L., POMMERENKE, W. T.: Cyclic fluctuation of the alveolar carbon dioxide tension during the normal menstrual cycle. Fertil. Steril. **3**, 394 (1952).

GREENBLATT, R. B.: One-pill-a-month contraceptive. Fertil. Steril. **18**, 207 (1967).

— Antiovulatory drugs and indications for their use. Med. Clin. N. Amer. **45**, 973 (1961).

— Ovulation. Philadelphia: Lippincott 1966.

GUAL, C.: Pregnandiol studies in the detection of ovulation. In: Ovulation, GREENBLATT, R. B., Ed. Philadelphia: Lippincott 1966.

HABERLANDT, L.: Über hormonale Sterilisierung des weiblichen Tierkörpers. Münch. med. Wschr. **68**, 1577 (1921).

HALLER, J.: Ovulationshemmung durch Hormone. Stuttgart: Thieme 1968.

HARTMANN, C.: How variable is the functional life of the human corpus luteum menstruationis? Wld. Obstet. Gynec. **7**, 323 (1955).

— Science and the safe period. Baltimore: Williams & Wilkins 1962.

HERTIG, A. T.: Proc. Conf. on Diagnosis in Sterility. Springfield (Ill.): Thomas 1946.

HERTZ, R., TULLNER, W., RAFFELT, E.: Progestational activita of orally administered 17-ethinyl-19-nortestosterone. Endocrinology **54**, 228 (1954).

HERVE, R., ROBEY, M., SERGENT, P.: Analyse immunodectroparetique du mucus cervical. Gynéc. Obstét. **64**, 347 (1965).

JAVERT, C. T.: Spontaneous and habitual abortion. New York: McGraw-Hill 1957.

JEFFERIES, W. MCK.: Glucocorticoids and ovulation. In: Ovulation, R. B. GREENBLATT, Ed., p. 62. Philadelphia: J. B. Lippincott Comp. 1966.

JOHANNISON, E., GEMZELL, C. A., DICZFALUSY, E.: Effects of a single injection of human pituitary follicle-stimulating hormone on urinary oestrogens and the vaginal smear in amenorrhoeic women. J. clin. Endocr. **21**, 1068 (1961).

INMAN, W. H. W., VESSEY, M. P.: Investigation of deaths from pulmonary and cerebral thrombosis and embolism in women of childbearing age. Brit. med. J. **1968 II**, 193.

— — WESTERHOLM, B., ENGELUND, A.: Thromboembolic disease and the steroidal content of oral contraceptives. A Report to the committee on safety of drugs. Brit. med. J. **1970 II**, 203.

KLOPPER, A. I.: The excretion of pregnandiol during the normal menstrual cycle. J. Obstet. Gynaec. Brit. Emp. **64**, 504 (1957).

KNAUS, K.: Die periodische Fruchtbarkeit und Unfruchtbarkeit des Weibes. In: Der Weg zur natürlichen Geburtenregelung, 2nd ed. Wien: Maudrich 1935.

KOTZ, H. L., HERRMANN, W.: A review of the endocrine induction of human ovulation. Fertil. Steril. **12**, 96 (1961).

KUPPERMAN, H. S.: Modern endocrine approach to the infertile patient. Postgrad. Med. **26**, 659 (1959).

LAURITZEN, CH.: Das Klimakterium. Med. Mitt. (Schering) **29**, 2 (1968).

LIGGINS, G. C.: Effect of variation in estrogen dosage on the pregnancy rate during sequential oral contraception. Fertil. Steril. **18**, 191 (1967).

LLOYD, C. W.: Human reproduction and sexual behavior. Philadelphia: Lea 1964.

LORAINE, J. A., BELL, E. T., HARKNESS, R. A., MEARS, E., JACKSON, M.: Hormone excretion patterns during and after the long-term administration of oral contraceptives. Acta endocr. (Kbh.) **50**, 15 (1965).

LUNENFELD, B., SULIMOVICI, S., RABAU, E.: Mechanism of action of antiovulatory compounds. J. clin. Endocr. **23**, 391 (1963).

MAKEPIECE, A. W., WEINSTEIN, G. L., FRIEDMAN, M. H.: The effect of progestin and progesterone on ovulation in the rabbit. Amer. J. Physiol. **119**, 512 (1937).

MANDL, A.M.: Factors influencing ovarian sensitivity to gonadotropins. J. Endocr. **15**, 448 (1957).
MARTINEZ-MANAUTOU, J., RUDEL, H.W.: Low dose contineous progestogen therapy. Presented at the 5th World Congress on Fertility and Sterility, Stockholm 1966.
MASTROIANNI, L.: Ovulation. Clin. Obstet. Gynec. **10**, 345 (1967).
MATSUMOTO, S., ITO, T., INOUE, S.: Untersuchungen der ovulationshemmenden Wirkung von 19-Norsteroiden an laparatomierten Patientinnen. Geburtsh. Frauenheilk. **20**, 250 (1960).
MCARTHUR, J.W.: Mid-cycle changes in urinary gonadotropin excretion. In: Recent progress in the endocrinology of reproduction, C.W. LLOYD, Ed., p. 59. New York: Acad. Press 1959.
— WORCESTER, J., INGERSOLL, F.M.: The urinary excretion of JCSH- and FSH-activity during the normal menstrual cycle. J. clin. Endocr. **18**, 1186 (1958).
MENON, K.: Trials with Enovid. Proc. 7th Int. Conf. Planned Parenthood Fed. Singapore 1963. Excerpta med., Int. Congr. Ser. **72**, 355 (1963).
MOGHISSI, K.S.: Cyclic changes of cervical mucus proteins. Int. J. Fertil. **11**, 284 (1966).
MORICARD, R., FERIN, J.: L'ovulation. Paris: Masson 1969.
MORRIS, J.: Studies on inhibition of implantation of the fertilized ovum. Presented at the 5th World Congress on Fertility and Sterility. Stockholm, 1966.
MORRIS, J.MC L., WAGENEN, G.VAN, HURTEAU, G.D., JOHNSON, D.W., CARLSEN, R.A.: Compounds interfering with ovum implantation and development. I. Alkaloids and antimetabolites. Fertil. Steril. **18**, 7 (1967).
MÜNZNER, H., LOER, K.: Statistische Untersuchungen über die Abhängigkeit der weiblichen Fruchtbarkeit vom Alter. Zbl. Gynäk. **58**, 2900 (1934).
NOYES, R.W.: Uniformity of secretory endometrium, multiple sections removed from 100 uteri. Fertil. Steril. **7**, 103 (1956).
— HAMAN, J.O.: The accuracy of endometrial dating. Fertil. Steril. **4**, 504 (1953).
— HERTIG, A.T., ROCK, J.: Dating the endometrial biopsy. Fertil. Steril. **1**, 3 (1950).
PAPANICOLAOU, G.: A specific inhibitory hormone of the corpus luteum. Its contrasts with the female sex (follicular) hormone. J. Amer. med. Ass. **86**, 1422 (1926).
PARKES, A.S., BRUCE, H.M.: Olfactory stimuli in mammalian reproduction. Science **134**, 1049 (1961).
PHILLIPS, W.A.: The inhibition of oestrus cycles in the albino rat by progesterone. Amer. J. Physiol. **119**, 623 (1937).
PINCUS, G.: Some effects of progesterone and related compounds upon reproduction and early development in mammals. Proc. 5th Intern. Conf. Planned Parenthood, p. 175, Tokyo Japan 1955.
— Long-term administration of Enovid to human subjects. In: Proc. Sympos. on 19-nor-progestational Steroids, p. 105. Chicago: Searle 1957.
— The control of fertility. New York: Academic Press 1965.
— CHANG, M.C.: The effects of progesterone and related compounds on ovulation and early development in the rabbit. Acta physiol. lat.-amer. **3**, 177 (1953).
PSCHYREMBEL, W.: Praktische Gynaekologie, p. 581 ff. Berlin: W. de Gruyter & Co. 1968.
RATNER, R., MEITES, J.: The removal of prolactin-inhibiting activity of the rat hypothalamus by estrogens, reserpine or suckling. Fed. Proc. **23**, 110 (1964).
RAUSCHER, H.: Vergleichende Untersuchungen über das Verhalten des Vaginalabstrichs, der Zervixfunktion und der Basaltemperatur in zweiphasischen Zyklen. Geburtsh. Frauenheilk. **14**, 327 (1954).
— Die Ermittlung der praeovulatorischen Phase durch die Simultanuntersuchung von Vaginalabstrich und Cervix. Geburtsh. Frauenheilk. **16**, 850 (1956).
— Répercussion du cycle ovarien sur le col utérin et la sécrétion cervicale. In: Les fonctions du col utérin. Paris: Masson 1964.
RICE-WRAY, E.: Field study with Enovid as a contraceptive agent. In: Proc. Sympos. on 19-nor-progestational steroids, p. 78. Chicago: Searle 1957.
— Ovulation inhibition with longer acting injectable steroids. Presented at the 6th Pan-American Congress of Endocrinology, Mexico City, Oct. 15, 1965.
RICHTER, R.H.H., SCHREINER, W.E.: Die temporäre hormonale Sterilisierung der Frau und ihre Problematik. Ther. Umsch. **20**, 489 (1963).
RILEY, G.M., DONTAS, E., GILL, B.: Use of serial vaginal smears in detecting the time of ovulation. Fertil. Steril. **6**, 86 (1955).
ROCK, J., BARTLETT, M.: Biopsy studies of human endometrium. J. Amer. med. Ass. **108**, 2022 (1937).
— GARCIA, C.R., PINCUS, G.: Effects of certain 19-norsteroids on the normal human menstrual cycle. Science **124**, 891 (1956).
— — — Synthetic progestins in the normal human menstrual cycle. Recent Progr. Hormone Res. **13**, 323 (1957).
ROGERS, J.: Endocrine and metabolic aspects of gynecology. Philadelphia: Saunders 1963.
ROSEMBERG, E., KELLER, P.J.: Studies on the urinary excretion of FSH- and LH-activity during the menstrual cycle. J. clin. Endocr. **25**, 1262 (1965).
Royal College of General Practitioners: Oral contraception and thromboembolic disease; a report by the records unit and research advisory service of the Royal College of General Practitioners. J. Coll. gen. Practit. **13**, 267 (1967); Brit. med. J. **1967 II**, 355.
RUDEL, H.W., MARTINEZ-MANAUTOU, J., MAGUEO-TOPETE, M.: The role of progesteron in the hormonal control of fertility. Fertil. Steril. **16**, 158 (1965).
SABIN, M., LATOUR, J.P.A.: Test for the rapid diagnosis of secretory endometrium. Fertil. Steril. **5**, 44 (1954).
SAN, C.C.: Clinical trials on oral contraceptives in Singapore family planning association. Proc. 7th Int. Conf. Planned Parenthood Fed. Singapore, 1963. Excerpta med. Int. Congr. Ser. **72**, 382 (1963).
SCHNEEBERG, N.G.: The thyroid gland and menstrual disorders. In: Diagnosis and treatment of menstrual disorders and sterility, ISRAEL, S.L., Ed. 5th ed., p. 275, New York: Hoeber 1967.
SCHREINER, W.E.: Wirkungen und Nebenwirkungen der Ovulostatika. Praxis **55**, 94 (1966).
SÉGNY, J., SIMMONET, H.: Recherches de signes directs d'ovulation chez la femme. Gynéc. Obstét. **28**, 657 (1933).
STARR, P.: Subclinical hypothyroidism, recognition and treatment. Calif. Med. **97**, 263 (1962).
— The effect of thyroxine on ovulation and associated mechanisms. In: Ovulation, GREENBLATT, R.B., Ed. Philadelphia: Lippincott 1966.
STEVENS, V.C., VORYS, N.: The effect of various compounds on urinary FSH and LH excretion. Symp. on Recent Advanc. Ovarian and Synthetic Steroids, Sydney, Australia 1965, p. 49.
STURGIS, S.H. (Ed.): The gynecologic patient. New York and London: Grune & Stratton 1962.
SWAAB, L.I.: A field trial on Lyndiol and Enovid. Proc. 7th Int. Conf. Planned Parenthood Fed. Singapore 1963. Excerpta med. Int. Congr. Ser. **72**, 369 (1963).
TAYMOR, M.L., PLANCK, S., YAHIA, C.: Ovulation inhibition with a long acting parenteral progestin-estrogen combination. Fertil. Steril. **6**, 653 (1964).
VELDE, T.H., VAN DE: Über den Zusammenhang zwischen Ovarialfunktion, Wellenbewegung und Menstrualblutung und über die Entstehung des sog. Mittelschmerzes, p. 39. Haarlem: Bohn 1905.
VENNING, G.R.: The influence of contraceptive practice upon maternal and child health. Metabolism **14**, 457 (1965).

VESSEY, M.P., DOLL, R.: Investigation of relation between use of oral contraceptives and thromboembolic disease. Brit. med. J. **1968II**, 199.
VOLLMANN, R., VOLLMANN, U.: Vergleichende Temperaturuntersuchungen zur Reproduktionsphysiologie der Frau und der Kuh. Schweiz. Arch. Tierheilk. **84**, 403, 450 (1942).
WALLACH, E.E.: Breast and reproductive system effects of oral contraceptives. Clin. Obstet. Gynec. **11**, 645 (1968).
— WATSON, F.M., Jr., GARCIA, C.R.: Patient acceptance of oral contraceptives. II. The private patient. Amer. J. Obstet. Gynec. **98**, 1071 (1967).
WIDE, L.: Immunoassay of human LH for the detection of ovulation. In: Ovulation, GREENBLATT, R.B., Ed., p. 283. Philadelphia: Lippincott 1966.
— GEMZELL, C.: Immunological determination of pituitary luteinizing hormone in the urine of fertile and postmenopausal women and adult men. Acta endocr. (Kbh.) **39**, 539 (1962).
WYLER: Das Höchstalter der Gebärfähigkeit der Frau. Schweiz. med. Wschr. **87**, 689 (1957).
ZAFFARONI, A.: Steroids, LXIII. Synthesis of Δ^4-19-norpregnene and related 19-noradrenal hormones. J. Amer. chem. Soc. **76**, 6210 (1954).

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ABRAMS, C.A.L., GRUMBACH, M.M., DYRENFURTH, I., VAN DE WIELE, R.L.: Ovarian stimulation with human menopausal and chorionic gonadotropins in a prepubertal hypophysectomized female. J. clin. Endocr. **27**, 467 (1967).
ALBERT, A., MENDOZA, D.: Daily fluctuation in excretion of follicle stimulating and luteinizing hormones by a postmenopausal woman. J. clin. Endocr. **26**, 371 (1966).
BACKMAN, G.: Die beschleunigte Entwicklung der Jugend. Acta anat. (Basel) **4**, 421 (1948).
BÄTTIG, A.: Genitalblutungen in der Menopause. Inaug. Diss. Zurich 1968.
BAHNER, F.: Fettsucht und Magersucht. In: Handbuch der inneren Medizin, 4th ed., Bd. VII/1, BERGMANN, G., W. FREY, H. SCHWIEGK, Eds. Berlin-Heidelberg-New York: Springer 1955.
BANERJIE, D., MUKHERJEE, S.P.: Menarche in Bengalee hindu girls. J. Indian med. Ass. **37**, 261 (1961).
BARRETT, M.S.: An investigation of the menopause in one thousand women. Lancet **1933I**, 106.
BÉCLÈRE, C.: Les dosages hormonaux sous les troubles de la préménopause et de la postménopause. Assise franç. de Gynéc. Nice, Mai rapports M. Destrons 43 (1963).
BENJAMIN, F.: Age of menarche and of the menopause in white south African women and certain factors influencing these times. S. Afr. med. J. **34**, 316 (1960).
BENNHOLDT-THOMSEN, C.: Die Entwicklungsbeschleunigung der Jugend. Ergebn. inn. Med. Kinderheilk. **62**, 1153 (1942).
BERKSON, D.M., STAMLER, J., COHEN, D.B.: Ovarian function and coronary atherosclerosis. Clin. Obstet. Gynec. **7**, 504 (1964).
BETTENDORF, G., APOSTOLAKIS, M., VOIGT, K.D.: Darstellung von Gonadotropinen aus menschlichen Hypophysen. Acta endocr. (Kbh.) **41**, 1 (1962).
BLOCK, E.: Quantitative morphological investigations of the follicular system in women. Variations at different ages. Acta anat. (Basel) **14**, 108 (1952).
BREWER, J.I., MILLER, W.H.: Postmenopausal uterine bleeding. Amer. J. Obstet. Gynec. **67**, 988 (1954).
BROWN, J.B., KELLAR, R., MATTHEW, G.D.: Preliminary observations on urinary oestrogen excretion in certain gynaecological disorders. J. Obstet. Gynaec. Brit. Emp. **66**, 177 (1959).
BULBROOK, R.D., GREENWOOD, F.D.: The persistence of oestrogen production after endocrine ablation. Acta endocr. (Kbh.), Suppl. **31**, 324 (1957).
— — Persistence of urinary estrogen excretion after oophorectomy and adrenalectomy. Brit. med. J. **1957I**, 662.
— — HADFIELD, G.J., SCOWERS, E.F.: Oophorectomy in breast cancer. An attempt to correlate clinical results with oestrogen production. Brit. med. J. **1958II**, 7.
DALY, M.J.: Physical and psychological development of the adolescent female. Clin. Obstet. Gynec. **9**, 711 (1966).
DAVIS, M.E.: The physiology and management of the menopause. In: Advances in obstet. gynec., MARCUS, ST.L., MARCUS, C.C., Eds. Baltimore: Williams & Wilkins 1967.
DENNING, J.: Application of the compertz curve to the observed pattern of growth in length of 48 individual boys and girls during the adolescent cycle of growth. Hum. Biol. **29**, 83 (1957).
DEUTSCH, H.: Psychologie der Frau. Berne: H. Huber 1948.
DICZFALUSY, E., LAURITZEN, CH.: Oestrogene beim Menschen. Berlin-Göttingen-Heidelberg: Springer 1961.
DÖRING, G.K.: Über Ovulationsstörungen als Sterilitätsursache. Med. Welt (Stuttg.) **1959**, 403.
DONOVAN, B.T., WERFFTEN, J.J. VAN DER, BOSCH: Physiology of puberty. London: Arnold 1965.
ELLIS, R.W.B.: Age of puberty in the tropics. Brit. med. J. **1950I**, 85.
ENGLE, E.T.: Aspects of aging as reflected in the human ovary and testis. Recent Progr. Hormone Res. **11**, 291 (1955).
FROMMER, D.J.: Changing age of menopause. Brit. med. J. **1964II**, 349.
FURUHJELM, M.: Urinary excretion of hormones during the climacteric. Acta obstet. gynec. scand. **45**, 352 (1966).
GOECKE, H.: Die Klinik des Klimakteriums. Arch. Gynäk. **193**, 33 (1959).
GOLDFARB, A.F.: Puberty. Clin. Obstet. Gynec. **11**, 769 (1968).
GRIMM, H.: Der gegenwärtige Verlauf der Pubertät bei der weiblichen Berufsjugend Mitteldeutschlands. Zbl. Gynäk. **70**, 8 (1948).
HARRIS, G.S., JACOBSOHN, D.: Functional hypophyseal grafts. Ciba Found. Coll. Endocr. **4**, 115 (1952).
HAUSER, G.A., OBIRI, J.A., VALAER, M., ERB, H., MÜLLER, TH., REMEN, U., VANÄÄNEN, P.: Der Einfluß des Menarchealters auf das Menopausealter. Gynaecologia (Basel) **152**, 279 (1961).
— WENNER, R.: Das Klimakterium der Frau. Ergebn. inn. Med. Kinderheilk. **16**, 125 (1961).
HELD, E., SCHREINER, W.E., OEHLER, I.: Bedeutung der Kolposkopie und Cytologie zur Erfassung des Genitalkarzinoms. Schweiz. med. Wschr. **84**, 856 (1954).
HELLER, C.G.: Josiah Macy jr. Found. 7th J. Meeting, p. 60 (1944); cited by DICZFALUSY, E., Oestrogene beim Menschen. Berlin-Göttingen-Heidelberg: Springer 1961.
HENTON, C.L.: Comparative study of the onset of menarche among Negro and white children. J. psychol. **46**, 65 (1958).
HERTIG, A.T.: The aging ovary. J. clin. Endocr. **4**, 581 (1944).
HOFF, F.: Klimakterium und innere Medizin. Arch. Gynäk. **193**, 12 (1959).
ISRAEL, S.L.: Diagnosis and treatment of menstrual disorders and sterility. New York: Hoeber 1967.
ITO, P.K.: Comparative biometrical study of physique of Japanese women born and reared under different environments. Hum. Biol. **14**, 279 (1942).
JAVERT, C.T.: Spontaneous and habitual abortion. New York-Toronto-London: McGraw-Hill 1957.
JOHNSON, S.G.: A clinical routine-method for the quantitative determination of gonadotropins in 24-hour-urine samples, II: Normal values for men and women at all age groups from pre-puberty to senescence. Acta endocr. (Kbh.) **31**, 209 (1959).
KAISER, R., DAUME, D.: Über eine einheitliche Nomenklatur für das Klimakterium und seine Begleitsymptome. Geburtsh. Frauenheilk. **25**, 974 (1965).
KEETTEL, W.C., BRADBURY, J.T.: Premature ovarian failure, permanent and temporary. Amer. J. Obstet. Gynec. **89**, 83 (1964).

KELLER, P.J.: Die Bedeutung der Gonadotropin-Bestimmung für Diagnose und Therapie der Sterilität. Vortrag gynäk. Fortbildungskurs, Liestal 1968.
— Hypophysäre Gonadotropine: Untersuchungen über Physiologie, Pathologie und medikamentöse Beeinflussung der FSH- und LH-Sekretion bei der Frau. Habilitation, Zürich 1970.
KUPPERMAN, H.S., WETCHLER, B.B., MEYER, H.G.: Contemporary therapy of the menopausal syndrome. J. Amer. med. Ass. **171**, 1627 (1959).
LAJOS, L., GÖRCS, J., ILLEI, G., KECSKÉS, L., MUTSCHLER, F., GLOS, I.: Über den klimakterischen Hyperoestrogenismus. Z. Geburtsh. Gynäk. **159**, 308 (1963).
LAURITZEN, CH.: Das Klimakterium. Med. Mitteilungen (Schering) **29**, 2 (1968).
LEE, M.M.C., CHANG, K.S.F., CHAN, M.M.C.: Sexual maturation of Chinese girls in Hong Kong. Pediatrics **32**, 389 (1963).
LENZ, W., KELLNER, H.: Die körperliche Akzeleration. Ed. Deutsches Jugendinstitut. Munich: Juventa 1965.
LEVINE, V.E.: Studies in physiological anthropology. III. The age of onset of menstruation of the Alaska Eskimos. Amer. J. phys. Anthrop., N.S. **11**, 252 (1953).
LYNDA, L.E.: Gynecologic endocrinology of adolescence. Clin. Obstet. Gynec. **9**, 759 (1966).
MANDL, A.M., ZUCKERMAN, A.: Factors influencing the onset of puberty in albino rats. J. Endocr. **8**, 357 (1952).
MARANON, G.: The climacteric. St. Louis: Mosby 1929.
MATTINGLEY, R.F., HUANG, W.Y.: Steroidogenesis of the menopausal and postmenopausal ovary. Amer. J. Obstet. Gynec. **103**, 679 (1969).
MAYER, A.: Klimakterium. In: Biologie u. Pathologie des Weibes, SEITZ, L., A.I. AMREICH, Bd. I, gen. Teil I, 2nd ed. Munich: Urban & Schwarzenberg 1952.
MC BRIDE, J.M.: The normal postmenopausal endometrium. Excerpta med. (Amst.), Sect. X **9**, 37 (1956).
MENDUKE, H., LEIB, J.G.: Statistics on the aging female in the United States. Clin. Obstet. Gynec. **10**, 445 (1967).
MORETTI, I.: Caratteri patologici concordanti in due gemelle monovulari: cataratta dell'occhio sinistro e degenerazione microcistica dell'ovaria destra. Folia hered. path. (Pavia) **3**, 3, H. 2 (1953).
MÜNZNER, H., LOER, K.: Statistische Untersuchungen über die Abhängigkeit der weiblichen Fruchtbarkeit vom Alter. Zbl. Gynäk. **58**, 2900 (1934).
PAPANICOLAOU, A.D., LORAINE, J.A., DOVE, G.A.: Endocrine function in postmenopausal women. J. Obstet. Gynaec. Brit. Cwlth **76**, 317 (1969).
— — — LOUDON, N.B.: Hormone excretion patterns in perimenopausal women. J. Obstet. Gynaec. Brit. Cwlth **76**, 308 (1969).
PASCHKIS, K.E., RAKOFF, A.E.: Clinical endocrinology. In: The hormones, G. PINCUS, K.V. THIMANN, Eds., vol. 3, p. 821. New York: Academic Press 1955.
PAULSEN, C.A., LEACH, R.B., SANDBERG, H., SHEINFELD, S., MADDOCK, W.O.: Function of the postmenopausal ovary; comparison of urinary estrogen and gonadotropin excretion and response to administration of FSH in postmenopausal and ovariectomized women. J. Amer. Geriat. Soc. **6**, 803 (1958).
PETER, R.: Gynäkologie des Kindesalters. In: Klinik der Frauenheilkunde und Geburtshilfe, Bd. I, p. 161, H. SCHWALM, G. DOEDERLEIN, Eds. Munich: Berlin: Urban & Schwarzenberg 1964.
PETRI, E.: Untersuchungen zur Erbbedingtheit der Menarche. Z. Morph. Anthrop. **33**, 43 (1935).
PLOTZ, E.J., FRIEDLANDER, R.L.: Endocrinology in women over 65 years of age. Clin. Obstet. Gynec. **10**, 466 (1967).
PORTMANN, A.: Kritische Betrachtungen zum Thema „Akzeleration". Lit. Eild. „Roche" **35**, 1 (1967).
POTTER, E.L.: The ovary in infancy and childhood. In: The ovary, H.G. GRADY, D.E. SMITH, Eds. Baltimore: Williams & Wilkins 1963.
PRILL, H.J.: Klimax praecox, Klimax tarda (ein statistischer Vergleich). Geburtsh. Frauenheilk. **26**, 883 (1966).
PRILL, H.J.: Klimax praecox, Klimax tarda (ein statistischer Vergleich). Geburtsh. u. Frauenheilk. **26**, 883 (1966).
PSCHYREMBEL, W.: Praktische Gynäkologie. Berlin: W. de Gruyter 1968.
PUNDEL, I.P.: Acquisition recentes en cytologie vaginale hormonale. Paris: Masson 1957.
REIFENSTEIN, E.C., JR.: Die Endokrinologie des alternden Menschen. 5. Sympos. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1958.
REYNOLDS, E.L., SCHOEN, G.: Growth patterns of identical triplets from 8 through 18 years. Child Develop. **18**, 130 (1947).
RÖSSLE, R., ROULET, F.: Maß und Zahl in der Pathologie. Berlin-Göttingen-Heidelberg: Springer 1941.
SCHÄFFER, R.: Über Beginn, Dauer und Erlöschen der Menstruation. Mschr. Geburtsh. Gynäk. **23**, 169 (1906).
— Über das Alter des Menstruationsbeginns. Arch. Gynäk. **84**, 657 (1908).
SCHULZ, F.H.: Die Wechselbeziehungen zwischen Klimakterium und innern Krankheiten. In: Die Endokrinologie des alternden Menschen. 5. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1958.
SCHWENK, A.: Die körperliche Entwicklung im Jugendalter und ihre endokrinologischen Grundlagen. Basel: Karger 1965.
SHARMAN, A.: The menopause. In: The ovary, S. ZUCKERMAN, Ed., vol. I, p. 539. New York, London: Academic Press 1962.
SMOLKA, H., SOOST, H.J.: Grundriß und Atlas der gynaekologischen Zytodiagnostik. Stuttgart: Thieme 1956.
SOHMA, M.: Über die Histologie der Ovarialgefäße in den verschiedenen Lebensaltern mit besonderer Berücksichtigung der Menstruations- und Ovulationssklerose. Arch. Gynäk. **84**, 377 (1908).
SOUTHAM, A.L.: Disorders of menstruation. Clin. Obstet. Gynec. **9**, 779 (1966).
— DAUGELA, M.: Delayed menarche. In: The management of amenorrhea, J. COLLINS, Ed. Springfield (Ill.): C. Thomas 1966.
STAEMMLER, H.J.: Die gestörte Regelung der Ovarialfunktion. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
STUART, H.C.: Normal growth and development during adolescence. New Engl. J. Med. **234**, 666, 693, 732 (1946).
TANNER, J.M.: Puberty. In: Advances in reproductive physiology, A. MC LAREN, Ed., Ch. 8, p. 311. London: Logos Press and New York: Academic Press 1967.
— Wachstum und Reifung des Menschen. Stuttgart: Thieme 1962.
— Earlier maturation in man. Sci. Amer. **218**, 21 (1968).
THOMAS, H.E., KANNEL, W.B., DONOVER, T.R., MCNAMARA, P.M.: Cholesterol-phospholipid ratio in the prediction of coronary heart disease. The Framingham study. New Engl. J. Med. **274**, 701 (1966).
TIETZE, K.: Der weibliche Zyklus und seine Störungen. In: SEITZ-AMREICH, Biologie und Pathologie des Weibes, Bd. II, p. 491. Munich: Urban & Schwarzenberg 1952.
TRIASSAC, R., PERRIN, D., MORRIN, J.: La ménopause chirurgicale. Assise franç. gynéc. Nice, 1956, rapports M. DESHONS, p. 179.
WAARD, F. DE, BURGT, A. TH. VAN DER: The age of menopause. Ned. T. Geneesk. **110**, 289 (1966).
WAGNER, H.: Das Klimakterium der Frau. Stuttgart: Enke 1955.
WALLACH, E.E.: Female isosexual pseudoprecocious puberty. Clin. Obstet. Gynec. **11**, 795 (1968).
WATZKA, M.: Weibliche Genitalorgane, das Ovarium. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. VII/3, MÖLLENDORFF, W., W. BARGMANN, Eds. Berlin-Göttingen-Heidelberg: Springer 1957.

WEGELIN, C.: Über Arteriosklerose im Myokard. Schweiz. med. Wschr. **74**, 57 (1944).
WELLENBACH, B.: Childhood gynecologic endocrinology. Ann. N.Y. Acad. Sci. **142**, 592 (1967).
WENNER, R., HAUSER, G.A.: Neurovegetative Untersuchungen und Therapie — Ergebnisse bei klimakterischen Frauen. Arch. Gynäk. **193**, 58 (1959).
WERFF TEN BOSCH, J.J. VAN DER: Hypothalamic control of ovulation. Philadelphia: Lippincott 1966.
WIESEL, J.: Innere Klinik des Klimakteriums. In: Biologie und Pathologie des Weibes, HALBAN, J., L. SEITZ, Eds. Bd. 3, p. 1025. Berlin: Urban & Schwarzenberg 1924.
WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence. Springfield (Ill.): Thomas 1965.
WINTZ, H.: Über Ausfallserscheinungen und ihre Bewertung im spontan eingetretenen Klimakterium. Strahlentherapie **54**, 583 (1953).
WYLER, J.: Das Höchstalter der Gebärfähigkeit der Frau. Schweiz. med. Wschr. **87**, 698 (1957).
ZACHARIAS, L., WURTMAN, R.J.: Blindness: Its relation to age of menarche. Science **144**, 1154 (1964).
ZONDEK, B.: Oestrogens and tumour genesis. Acta radiol. (Stockh.) **28**, 433 (1947).

Impaired Ovarian Function

Amenorrhea

AHUMADA, J.C., CASTILLO, E.B. DEL: Amenorrea y Galactorrhea. Bol. Soc. obstet. ginec. B. Aires **11**, 64 (1932).
ARGONZ, J., CASTILLO, E.B. DEL: A syndrome characterized by estrogenic insufficiency, galactorrhoea, and decreased urinary gonadotropin. J. clin. Endocr. **13**, 79 (1953).
ASHERMAN, J.G.: Amenorrhea traumatica. J. Obstet. Gynaec. Brit. Emp. **55**, 23 (1948).
— Traumatic intrauterine adhesions. J. Obstet. Gynaec. Brit. Emp. **57**, 892 (1950).
— Traumatic intrauterine adhesions and the effect on fertility. Int. J. Fertil. **2**, 49 (1957).
BÄBLER, M.: Die operative Behandlung der kongenitalen Vaginalaplasie. Dissertation, Zürich 1967.
BAHNER, F.: Die allgemeine Konstitution des weiblichen Organismus, ihre Entwicklung und ihre Störungen. In: Gynäkologie und Geburtshilfe, KÄSER, O., Ed., Bd. I, p. 134. Stuttgart: Thieme 1969.
BARTON, M., WIESNER, B.P.: Thermogenetic effect of progesterone. Lancet **1945II**, 663, 671.
BASS, F.: L'aménorrhée au camp de concentration de Terezin (Theresienstadt). Gynaecologia (Basel) **123**, 211 (1947).
BÉCLÈRE, C., SIMMONET, H.: Le syndrome hyperhormonal hypophyseo-ovarien est une réalité. Presse méd. **71**, 3 (1963).
BENEDEK, TH.: Studies in pseudosomatic medicine. New York: Ronald 1952.
BERCOVICI, B., EHRENFELD, E.N.: Non puerperal galactorrhoea. J. Obstet. Gynaec. Brit. Cwlth **70**, 295 (1963).
BETTENDORF, G., APOSTOLAKIS, M.: Beeinflussung der hypogonadotropen Ovarialinsuffizienz durch hypophysäres Human-Gonadotropin. Geburtsh. Frauenheilk. **26**, 1281 (1966).
BICKERS, WM.: Amenorrhea and oligomenorrhea. Amer. J. Obstet. Gynec. **56**, 893 (1948).
BIEDL, A.: Geschwisterpaar mit adiposogenitaler Dystrophie. Dtsch. med. Wschr. **48**, 1630 (1922).
— Über das Laurence-Syndrom. Med. Klin. **29**, 839 (1933).
BLEULER, M.: Endokrinologische Psychiatrie. Stuttgart: Thieme 1954.
BLISS, E.L., BRAUCH, C.H.: Anorexia nervosa. New York: Hoeber 1960.
BRICAIRE, H., MOREAU, L., ELISSADE, B., BOUVIER, J.M.: Amenorrhea-galactorrhea syndrome associated with pituitary chromophobe adenoma (the Argonz-del Castillo or Forbes-Albright syndrome). Ann. Endocr. (Paris) **19**, 719 (1958).
BROWN, D.M., JENNESS, R., ULSTROM, R.A.: A study of the composition of milk from a patient with hypothyreoidism and galactorrhea. J. clin. Endocr. **25**, 1225 (1965).
BUSCHBECK, H.: Gibt es einen Rebound-Effekt bei der Frau? Medizinische **42**, 1523 (1957).
CANFIELD, M.C.J., BATES, R.W.: Non puerperal galactorrhoe. New Engl. J. Med. **273**, 897 (1965).
CHARD, T.: The Chiari-Frommel syndrome: An experiment of nature. J. Obstet. Gynaec. Brit. Cwlth **71**, 624 (1964).
CHIARI, J.: Anomalien der Größe. In: Klinik der Geburtshilfe und Gynäkologie, Chap. 16. Erlangen: Enke 1852.
CONDRAU, G.: Psychosomatik der Frauenheilkunde. Berne: Huber 1965.
CROOKE, A.C., BUTT, W.R., BERTRAND, P.W., MORRIS, R.: Treatment of infertility and secondary amenorrhoea with follicle-stimulating hormone and chorionic gonadotrophin. Lancet **1967II**, 636.
FORBES, A.P., HENNEMAN, P.H., GRISWOLD, G.C., ALBRIGHT, F.: Syndrome characterized by galactorrhea and low urinary FSH: Comparison with acromegaly and normal lactation. J. clin. Endocr. **14**, 265 (1954).
FRIED, P.H., RAKOFF, A.E., SCHOPBACH, R.R., KAPLAN, A.J.: Pseudocyesis: A psychosomatic study in gynecology. J. Amer. med. Ass. **145**, 1329 (1951).
FROMMEL, R.: Über puerperale Atrophie des Uterus. Z. Geburtsh. Gynäk. **7**, 305 (1882).
GEMZELL, C.A.: Induction of ovulation with human gonadotropins. Recent Progr. Hormone Res. **21**, 285 (1965).
— Ovulation. Mediz. Prisma **1**, 3 (1969).
GREENBLATT, R.B.: Induction of ovulation with Clomiphene. In: Ovulation, R.B. GREENBLATT, Ed. Philadelphia: Lippincott 1966.
GRIMM, H.: Der gegenwärtige Verlauf der Pubertät bei der weiblichen Berufsjugend Mitteldeutschlands. Zbl. Gynäk. **70**, 8 (1948).
GORDON, D.L., PAULSEN, C.A.: Premature menopause in mosaicism. Amer. J. Obstet. Gynec. **97**, 85 (1967).
GULL, W.W.: Anorexia nervosa (Apepsia hysterica, Anorexia hysterica). Trans. clin. Soc. Lond. **1**, 22 (1874).
GUMPEL, R.C.: Pituitary tumor, postpartum amenorrhea and galactorrhea with comment on Chiari-Frommel syndrome. N.Y. State J. Med. **60**, 3304 (1960).
HASKINS, A.L., MOZKOWSKI, E.F., COHEN, H.: Chiari-Frommel syndrome: Medroxyprogesterone acetate therapy. Amer. J. Obstet. Gynec. **88**, 667 (1964).
HAUSER, G.A.: Gonadendysgenesie. In: Die Intersexualität, OVERZIER, C., Ed., p. 304. Stuttgart: Thieme 1961.
— SCHREINER, W.E.: Das Mayer-Rokitansky-Küster-Syndrom. Schweiz. med. Wschr. **91**, 381 (1961).
HEYNEMANN, T.: Die Nachkriegsamenorrhoe. Klin. Wschr. **26**, 129 (1948).
HOHLWEG, W.: Med. Mittl. Schering-Kahlbaum AG **6**, 9 (1934); zit. BUSCHBECK, H., Medizinische **42**, 1523 (1957).
HOOPER, J.H., WELCH, V.C., SHACKELFORD, R.T.: Abnormal lactation associated with tranquilizing drug therapy. J. Amer. med. Ass. **178**, 166 (1961).
JACOBS, P.A., HARNDEN, D.G., BUCKTON, K.E., COURT BROWN, W.M., KING, M.J., MCBRIDE, J.A., MCGREGOR, T.N., MAC LEAN, N.: Cytogenetic studies in primary amenorrhoea. Lancet **1961I**, 1183.
JAGIELLO, G.M., ROGERS, J.: Amenorrhea and pituitary tumors. Fertil. Steril. **11**, 5 9 (1960).
JASZMANN, L.: The Chiari-Frommel syndrome. J. Obstet. Gynaec. Brit. Cwlth **70**, 120 (1963).
JONES, G.A.S.: The management of endocrine disorders of menstruation and fertility. Springfield (Ill.): Ch.C. Thomas 1954.
KEIFER, W.S., WORTHAM, J.T., ZANARTU, J., HAMBLEN, E.C.: The Laurence-Moon-Biedl syndrome: A confused symptom-complex. Amer. J. Obstet. Gynec. **60**, 72 (1950).

KELLER, P.J.: Excretion of follicle-stimulating and luteinizing hormone during lactation. Acta endocr. (Kbh.) **57**, 529 (1968).
— Ovulationsauslösung bei Zyklusstörungen. Schweiz. Z. Gynäk. Geburtsh. (in press).
— WYSS, H.J., NAVILLE, A.H.: Der Effekt einmaliger Gonadotropin-Applikation bei Anovulation. Gynaecologia (Basel) **167**, 434 (1969).
KEYS, A., BROZEK, J., HENSCHEL, A., MICHELSEN, O., TAYLOR, H.L.: The biology of human starvation. Univ. Minn. med. Bull. **1**, 749 (1950).
KINCH, R.A.H., PLUNKETT, E.R., SMOUT, M.S., CARR, D.H.: Primary ovarian failure: a clinico-pathological and cytogenetic study. Amer. J. Obstet. Gynec. **91**, 630 (1965).
KLEIN, J.J., SEGEL, R.L., WARNER, R.R.: Galactorrhea due to imipramine (Tofranil). Report of a case. New Engl. J. Med. **271**, 510 (1964).
KLINEFELTER, H.F., JR., ALBRIGHT, F., GRISWOLD, G.C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis. J. clin. Endocr. **3**, 529 (1943).
KNEER, M.: Amenorrhoe nach psychischem Trauma. Z. Geburtsh. Gynäk. **131**, 47 (1949).
KUMSCHIK, H., HAUSER, G.A.: Das diagnostische Vorgehen bei primärer Amenorrhoe. Schweiz. med. Wschr. **96**, 1055 (1966).
LASÈGUE, E.C.: De l'anorexie hystérique. Arch. gén. Méd. **21**, 385 (1873).
LORAINE, J.A., BILL, E.T., HARKNESS, R.A., HARRISON, M.T.: The effect of clomiphene on hormone excretion in patients with galactorrhoea. Acta endocr. (Kbh.) **52**, 527 (1966).
MACDONALD, D., O'DRISCOLL, K.: Suppression of lactation (a double blind trial). Lancet **1965II**, 623.
MAYER, A.: Über Menstruationsstörungen im Arbeitsdienst. Geburtsh. Frauenheilk. **7/8**, 457 (1948).
MCARTHUR, J.W., INGERSOLL, F.M., WORCESTER, J.: The urinary excretion of interstitial cell and follicle-stimulating hormone activity by women with disease of the reproductive system. J. clin. Endocr. **18**, 1202 (1958).
MCKUSICK, V.A., BAUER, R.L., KOOP, C.E., SCOTT, R.B.: Hydrometrocolpos as a simple inherited malformation. J. Amer. med. Ass. **189**, 813 (1964).
MENZER-BENARON, D., SABBATH, J., STURGIS, S.H.: Amenorrhea. In: The gynecological patient, STURGIS, S.H., Ed. New York: Grune & Stratton 1962.
MICHEL-WOLFROMM, H.: Gynécologie psychosomatique. Paris: Masson 1963.
MORRIS, J.: The syndrome of testicular feminization in male pseudohermaphroditism. Amer. J. Obstet. Gynec. **65**, 1192 (1953).
MOULTON, R.: The psychosomatic implications of pseudocyesis. Psychosom. Med. **4**, 376 (1942).
MÜLLER, P., SCHICK, R., LEVY, G.: Le syndrome amenorrhée-galactorrhée d'origine diencéphalique. C.R. Soc. franç. Gynéc. **32**, 557 (1962).
NEMIAH, J.C.: Anorexia nervosa: A clinical psychiatric study. Medicine (Baltimore) **29**, 225 (1950).
NETTER, A.: Sur la physiopathologie des amenorrhées des camps de concentration. Rev. franç. Endocr. **3**, 135 (1962).
NOCHIMOWSKI, J.: Die Ghettoamenorrhoe. Med. Klin. **41**, 347 (1946).
OVERZIER, C.: Echter Agonadismus. In: Die Intersexualität, OVERZIER, C., Ed., p. 348. Stuttgart: Thieme 1961.
— Die Intersexualität. In: Gynäkologie und Geburtshilfe. KÄSER, O., Ed., Bd. I, p. 93. Stuttgart: Thieme 1969.
PASCHKIS, K.E., RAKOFF, A.E.: Clinical endocrinology. In: The hormones, G. PINCUS, K.V. THIEMANN, Eds., vol. III, p. 821. New York: Academic Press 1955.
PHILIP, J., SELE, V., TROLLE, D.: Primary Amenorrhea: A study of 101 cases. Fertil. Steril. **16**, 795 (1965).
POLLOCK, G.H.: The psychologic response to estrogenic therapy in Turner's syndrome (Ovarian agenesis). J. nerv. ment. Dis. **121/5**, 420 (1955).
RABINOWITZ, P., FRIEDMAN, I.S.: Drug induced lactation in uremia. J. clin. Endocr. **21**, 1489 (1961).
RAKOFF, A.E.: Psychogenic factors in anovulatory women. I. Hormonal patterns in women with ovarian dysfunction of psychogenic origin. Fertil. Steril. **13**, 1 (1962).
ROCK, J.: Menstruation, its disorders and their treatment. New Engl. J. Med. **233**, 817 (1945).
ROGERS, J.: Endocrine and metabolic aspects of gynecology. Philadelphia: Saunders 1963.
SACHS, H.B.: Lactation after hysterectomy in a nulliparous woman. Amer. J. Obstet. Gynec. **78**, 204 (1959).
SCHREINER, W.E.: Über eine hereditäre Form von Pseudohermaphroditismus masculinus („testikuläre Feminisierung"). Geburtsh. Frauenheilk. **19**, 1110 (1959).
— Die Auslösung der Ovulation mit Clomid. Dtsch. med. Wschr. **23**, 1473 (1967).
SEITZ, L.: Wachstum, Geschlecht und Fortpflanzung. Berlin: Springer 1939.
STAEMMLER, H.J.: Klinik des hypoplastischen Ovarium. Arch. Gynäk. **198**, 377 (1963).
— Die gestörte Regelung der Ovarialfunktion. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
STANGE, H.H.: Geschwulstentwicklung bei gonadalen Fehlbildungen und ihre Bedeutung für das therapeutische Vorgehen. Zbl. Gynäk. **80**, 1503 (1958).
STEIN, I.F., LEVENTHAL, M.L.: Amenorrhea associated with bilateral polycystic ovaries. Amer. J. Obstet. Gynec. **29**, 181 (1935).
STRASSMANN, P.: Die Wiederherstellung des funktionierenden Canalis menstrualis. Zbl. Gynäk. **59**, 2871 (1935).
STROINK, J.A.: Amenorrhea in war time. Gynaecologia (Basel) **124**, 160 (1947).
STURGIS, S.H.: The gynecologic patient. New York: Grune & Stratton 1962.
SYDENHAM, A.: Amenorrhea at Stanley camp, Honkong during internment. Brit. med. J. **1946II**, 159.
THOMÄ, H.: Anorexia nervosa. Stuttgart: Huber & Klett 1961.
TIETZE, K.: Zur Genese und Prognose der Notstandsamenorrhoe. Zbl. Gynäk. **70**, 377 (1948).
WELLENBACH, B.: Childhood gynecologic endocrinology. Ann. N.Y. Acad. Sci. **142**, 592 (1967).
WHITEACRE, F.E., BARRERA, B.: War amenorrhea: A clinical and laboratory study. J. Amer. med. Ass. **124**, 399 (1944).
WIELE, R.L. VAN DE, TURKSOY, R.N.: Treatment of amenorrhea and of anovulation with human menopausal and chorionic gonadotropins. J. clin. Endocr. **25**, 369 (1965).
WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence. 2nd ed. Springfield: Ch.C. Thomas 1957.
WYK, J.J. VAN, GRUMBACH, M.M.: Syndrome of precocious menstruation and galactorrhea: An example of hormonal overlap in pituitary feedback. J. Pediat. **57**, 416 (1960).
ZANDER, J.: Diagnostik und Therapie der Amenorrhoe. Regensburg. Jb. ärztl. Fortbild. **12**, 136 (1964).
ZUBIRAN, S., GOMEZ-MONT, F.: Endocrine disturbances in chronic human malnutrition. Vitam. Horm. **11**, 97 (1953).

Dysfunctional Uterine Bleeding

ALLEN, W.M.: Functional uterine bleeding. Clin. Obstet. Gynec. **1**, 825 (1958).
AREY, L.B.: The degree of normal menstrual irregularity. An analysis of 20,000 calendar records from 1500 individuals. Amer. J. Obstet. Gynec. **37**, 12 (1939).
BEHRENS, H.: Histologische Studien am Endometrium als Grundlagen klinischer Diagnostik. Leipzig: VEB Georg Thieme 1956.
BREWER, J.I., JONES, H.O.: Studies on the human corpus luteum. Amer. J. Obstet. Gynec. **54**, 561 (1947).
BROMBERG, Y.M., BERCOVICI, B.: Occult intermenstrual bleeding about the time of ovulation. Fertil. Steril. **7**, 71 (1956).

BROWN, J.B., KELLER, R., MATTHEW, G.D.: Preliminary observations on urinary oestrogen excretion in certain gynaecological disorders. J. Obstet. Gynaec. Brit. Emp. **66**, 177 (1959).
DIDDLE, A.W.: Relation of intermenstrual symptoms and signs to ovulation as determined by basal body temperatures. Amer. J. Obstet. Gynec. **56**, 537 (1948).
DÖRING, G.K.: Über die relative Häufigkeit des anovulatorischen Zyklus im Leben der Frau. Arch. Gynäk. **199**, 115 (1963).
DEWHURST, D.J.: Gynaecological disorders of infants and children. London: Cassell 1963.
EFFKEMANN, G.: Die Pseudomenstruation als Sterilitätsursache bei der erwachsenen Frau. Zbl. Gynäk. **63**, 153 (1939).
FRIES, K.: Beitrag zur Kenntnis der glandulären Hyperplasie. Dissertation, Zürich 1947.
GUSBERG, S.B.: Developmental stages of uterine cancer and their diagnostic appraisal. Clin. Obstet. Gynec. **1**, 559 (1958).
HARTMAN, C.G.: Time of ovulation in women. Baltimore: Williams & Wilkins 1936.
HERTIG, A.T., ROCK, J.: A series of potentially abortive ova recovered from fertile women prior to the first missed menstrual period. Amer. J. Obstet. Gynec. **58**, 968 (1949).
— — ADAMS, E.: A description of 34 human ova within the first 47 days of development. Amer. J. Anat. **98**, 435 (1956).
ISRAEL, S.L.: Diagnosis and treatment of menstrual disorders and sterility, 5th ed. New York: Hoeber 1967.
KISTNER, R.W.: Gynecology, principles and practice. Year book Medical Publ. Inc., Chicago 1964, p. 237.
KNAUS, H.: Die Physiologie der Zeugung. Wien 1950.
LARSON, J.A.: Estrogens and endometrial carcinoma. Obstet. Gynec. **3**, 551 (1954).
LAX, H.: Die zu schwache Regelblutung. Geburtsh. Frauenheilk. **1**, 681 (1939).
MCCLENNAN, C.E.: Current concepts of prolonged or irregular endometrial shedding. Amer. J. Obstet. Gynec. **64**, 988 (1952).
MCKELVEY, J.L., SAMUELS, L.T.: Irregular shedding of the Endometrium. Amer. J. Obstet. Gynec. **53**, 627 (1947).
MEYER, R., RUGE, C.: Über Corpusluteum-Bildung und Menstruation in ihrer zeitlichen Zusammengehörigkeit. Zbl. Gynäk. **37**, 50 (1913).
MOSZKOWSKI, E., WOODRUFF, J.D., JONES, G.E.S.: The inadequate luteal phase. Amer. J. Obstet. Gynec. **83**, 363 (1962).
NOVAK, E.R., YUI, E.: Relation of endometrial hyperplasia to adenocarcinoma of uterus. Amer. J. Obstet. Gynec. **32**, 674 (1936).
OBER, K.G.: Grundlagen der Hormonbehandlung funktioneller gynäkologischer Blutungen. Dtsch. med. Wschr. **1955**, 532.
— Das Ovar. In: Klinik der inneren Sekretion, 1st ed., LABHART, A., Ed. Berlin-Göttingen-Heidelberg: Springer 1957.
SCHRÖDER, R.: Der mensuelle Genitalzyklus des Weibes und seine Störungen. In: Handbuch der Gynäkologie, V. VEIT, W. STOECKEL, Eds. Munich: Bergmann 1928.
— Über die Abgrenzung und Charakteristika der Hyperplasia endometrii. Congrès internat. de gynécologie et d'obstétrique, Genève 1954, p. 260.
SIEGLER, S., SIEGLER, A.M.: Evaluation of the basal body temperature. An analysis of 1012 basal body temperature recordings. Fertil. Steril. **2**, 287 (1951).
SOUTHAM, A.L.: Dysfunctional uterine bleeding in adolescence. Clin. Obstet. Gynec. **3**, 241 (1960).
— RICHART, R.M.: The prognosis for adolescents with menstrual abnormalities. Amer. J. Obstet. Gynec. **94**, 637 (1966).
SUTHERLAND, A.M.: Histology of the endometrium in "organic uterine haemorrhage". Lancet **1950 II**, 742.
— Functional uterine haemorrhage in puberty and adolescence. Glasg. med. J. **34**, 496 (1953).
TAYLOR, H.C., Jr.: Endometrial hyperplasia and carcinoma of the body of uterus. Amer. J. Obstet. Gynec. **23**, 309 (1932).
TE LINDE, R.W.: Endometrial pathology of functional bleeding. In: Menstruation and its disorders, E.T. ENGLE, Ed. Springfield, Ill.: Thomas 1950.
TRAUT, H.F., KUDER, A.: Irregular shedding and irregular ripening of endometrium. Surg. Gynec. Obstet. **61**, 145 (1935).
WEBER, E.: Beitrag zur Klinik des Corpuscarcinoms. Gynaecologia (Basel) **151**, 232 (1961).
ZONDEK, B.: On the mechanism of uterine bleeding. Amer. J. Obstet. Gynec. **68**, 310 (1954).

The Polycystic Ovary

ANDREWS, W.C., ANDREWS, M.C.: Stein-Leventhal syndrome with associated adenocarcinoma of the endometrium. Amer. J. Obstet. Gynec. **80**, 632 (1960).
AXELROD, L.R., GOLDZIEHER, J.W., ROSS, S.D.: Concurrent 3β-hydroxysteroid dehydrogenase deficiency in adrenal and sclerocystic ovary. Acta endocr. (Kbh.) **48**, 392 (1965).
BARLOW, J.J., LOGAN, C.M.: Estrogen metabolism in the polycystic ovary syndrome. Amer. J. Obstet. Gynec. **98**, 687 (1967).
BARRACLOUGH, C.A.: Production of anovulatory sterile rats by single injections of testosterone propionate. Endocrinology **68**, 62 (1961).
BARTUSKA, D.G., ESKIN, B.A., SMITH, E.M., DACOU, C., DRATMAN, M.B.: Brain damage, hypertrichosis and polycystic ovaries. Amer. J. Obstet. Gynec. **99**, 387 (1967).
BENEDICT, P.H., COHEN, R.B., COPE, O., SCULLY, R.E.: Ovarian and adrenal morphology in cases of hirsutism or virilism and Stein-Leventhal syndrome. Fertil. Steril. **13**, 380 (1962).
BISHUN, N.P., MORTON, W.R.M.: Chromosome mosaicism in Stein-Leventhal syndrome. Brit. med. J. **1964 II**, 1200.
BROWN, J.B., MATTHEW, G.D.: The application of urinary estrogen measurements to problems in gynecology. Rec. Progr. Hormone Res. **18**, 337 (1962).
BUTT, W.R., CROOKE, A.C., CUNNINGHAM, F., PALMER, R.: The effect of dexamethasone on the excretion of oestriol and FSH in patients with Stein-Leventhal syndrome. J. Endocr. **26**, 303 (1963).
BYRD, J.R., MAHESH, V.B., GREENBLATT, R.B.: Chromosomal studies in the Stein-Leventhal syndrome. J. clin. Endocr. **24**, 939 (1964).
CHAMBERLAIN, G., WOOD, C.: Stein-Leventhal syndrome. Brit. med. J. **1964 I**, 96.
CHOSSON, J., SERMENT, H., RUF, H., CODACCIONI, J.L.: Polycystose ovarienne et hyperandrogénie. Ann. Endocr. (Paris) **19**, 734 (1958).
DIGNAM, W., PION, R., LAMB, E., SIMMER, H.: Plasma androgens in women. II. Patients with polycystic ovaries and hirsutism. Acta endocr. (Kbh.) **45**, 254 (1964).
FORCHIELLI, E., SORCINI, G., PERLOFF, W.H., JACOBSON, L.G., NIGHTINGALE, M.S., BRUST, N., DORFMAN, R.I.: Testosterone in human plasma. Analyt. Biochem. **5**, 416 (1963).
GALLAGHER, T.F.: Steroid hormone metabolism and the control of adrenal secretion. Harvey Lect. **52**, 1 (1956/57).
GEMZELL, C.: Induction of ovulation with human gonadotropins. Recent Progr. Hormone Res. **21**, 179 (1965).
GOLD, J.J., FRANK, R.: The borderline adrenogenital syndrome: an intermediate entity. Amer. J. Obstet. Gynec. **75**, 1034 (1958).
GOLDZIEHER, J.W.: Polycystic ovarian disease. In: Advances in Obstet. Gynec., vol. I, p. 354, MARCUS, ST.L., C.C. MARCUS, Eds. Baltimore: Williams & Wilkins 1967.

— AXELROD, L. R.: Adrenal and ovarian steroidgenesis in the sclerocystic ovary syndrome. Acta endocr. (Kbh.), Suppl. **51**, 617 (1960).
— — The polycystic ovary. II. Urinary steroid excretion. J. clin. Endocr. **22**, 425 (1962).
— — Clinical and biochemical features of polycystic ovarian disease. Fertil. Steril. **14**, 631 (1963).
— GREEN, J. A.: The polycystic ovary. I. Clinic and histological features. J. clin. Endocr. **22**, 325 (1962).
— LAITIN, H.: Evaluation of the pituitary-adrenal axis in simple hirsutism. J. clin. Endocr. **20**, 967 (1960).
GREEN, J. A., GOLDZIEHER, J. W.: The polycystic ovary. IV. Light and electron microscope studies. Amer. J. Obstet. Gynec. **91**, 173 (1965).
GREENBLATT, R. B.: Cortisone in treatment of hirsute female. Amer. J. Obstet. Gynec. **66**, 700 (1953).
— BALDWIN, K. R.: The polycystic ovary syndrome (Stein-Leventhal syndrome). J. clin. Endocr. **1**, 498 (1960).
— CONIFF, R. F.: Hirsutism and the Stein-Leventhal syndrome. Fertil. Steril. **19**, 661 (1968).
INGERSOLL, F. M., MCARTHUR, J. W.: Longitudinal studies of gonadotropin excretion in the Stein-Leventhal syndrome. Amer. J. Obstet. Gynec. **77**, 795 (1959).
JAYLE, M. F., VEYRIN-FORRER, F., GELLER, S., MÈGE, F.: Biochemical classification of luteal insuffiencies with the aid of a new test for functional examination of the corpus luteum. Gynéc. Obstét. **60**, 381 (1961).
JEFFCOATE, T. N. A.: The androgenic ovary, with special reference to the Stein-Leventhal syndrome. Amer. J. Obstet. Gynec. **88**, 143 (1964).
JOHNSON, J. E.: Outcome of pregnancies following clomiphen citrate therapy. Presented at 5th world congress on fertility and sterility, Stockholm, 1966.
JONES, G. E. S., HOWARD, J. E., LANGFORD, H.: The use of cortisone in follicular phase disturbances. Fertil. Steril. **4**, 49 (1953).
— MORAES-RUEHSEN, M. DE: Induction of ovulation with human gonadotropins and with clomiphen. Fertil. Steril. **16**, 461 (1965).
JONES, H. W., JONES, G. E. S.: The gynecological aspects of adrenal hyperplasia and allied disorders. Amer. J. Obstet. Gynec. **68**, 1330 (1954).
KEETTEL, W. C.: Discussion. Amer. J. Obstet. Gynec. **77**, 1271 (1959).
KELLER, P. J.: Hormonal studies in endocrine disease. Germ. med. Mth. **13**, 1 (1968).
KISTNER, R. W.: Induction of ovulation with clomiphene citrate (Clomid). In: Progress in infertility, BEHRMAN, S. J., R. W. KISTNER, Eds. Boston: Little 1967.
— SMITH, O. W.: Observations on the use of a nonteroidal estrogen antagonist: MER-25. II. Effects in endometrial hyperplasia and Stein-Leventhal syndrome. Fertil. Steril. **12**, 121 (1961).
KORENMAN, S. G., KIRSCHNER, M. A., LIPSETT, M. B.: Testosterone production in normal and virilized women and in women with the Stein-Leventhal syndrome or idiopathic hirsutism. J. clin. Endocr. **25**, 798 (1965).
LANTHIER, A., SANDOR, T.: "*In-vitro*" production of androgenic steroids by human normal and "Stein-Leventhal type" ovarian slices. Preliminary report. Metabolism **9**, 861 (1960).
LEVENTHAL, M. L.: The Stein-Leventhal syndrome. Amer. J. Obstet. Gynec. **76**, 825 (1958).
— Functional and morphologic studies of the ovaries and suprarenal glands in the Stein-Leventhal syndrome. Amer. J. Obstet. Gynec. **84**, 154 (1962).
— SCOMMEGNA, A.: Multiglandular aspects of the Stein-Leventhal syndrome. Amer. J. Obstet. Gynec. **87**, 445 (1963).
LLOYD, C. W., LOBOTSKY, J., SEGRE, E. J., KOBAYASKI, T., TAYMOR, M. L., BATT, R. E.: Plasma testosterone and urinary 17-ketosteroids in women with hirsutism and polycystic ovaries. J. clin. Endocr. **26**, 314 (1966).
LOBOTSKY, J., WYSS, H. I., SEGRE, E. J., LLOYD, C. W.: Plasma testosterone in the normal women. J. clin. Endocr. **24**, 1261 (1964).
LUNENFELD, B., MANZI, A., VOLET, B.: Clinical effects of human postmenopausal gonadotrophin. First Internat. Congr. Endocrin. (Copenhagen) Abstr. **295**, 587 (1960).
MACDONALD, P. C., SIITERI, P. K.: Quantitation of estrogen production from circulating C_{19} precursors in nonpregnant subjects. Excerpta Medica, Internat. Congr. Ser. No 111, Abstract No 241 (1966).
MAHESH, V. B., GREENBLATT, R. B.: Isolation of dehydroepiandrosterone and 17α-hydroxy-Δ 5 pregnenolone from the polycystic ovaries of the Stein-Leventhal syndrome. J. clin. Endocr. **22**, 441 (1962).
— — Urinary steroid excretion patterns in hirsutism. II. Effect of ovarian stimulation with human pituitary FSH on urinary 17-ketosteroids. J. clin. Endocr. **24**, 1293 (1964).
— — AYDAR, C. K., ROY, S.: Secretion of androgens by the polycystic ovary and its significance. Fertil. Steril. **13**, 513 (1962).
MAUVAIS-JARVIS, P., BAULIEU, E.: Urinary 17-ketosteroid conjugates in hirsutism. J. clin. Invest. **41**, 1690 (1962).
MCGOOGAN, L. S.: Sterility and ovarian pathology. Obstet. Gynec. **3**, 254 (1954).
MIGEON, C. J.: Androgens in human plasma. In: Hormones in human plasma, H. N. ANTONIADES, Ed., p. 297. Boston: Little, Brown 1960.
MORRIS, J. MCL., SCULLY, R. E.: Endocrine pathology of the ovary. St. Louis: Mosby 1958.
NETTER, A., LAMBERT, A., HASKELES, M.: Syndrome de Stein-Leventhal. A propos de 4 cas. C. R. Soc. franç. Gynéc. **28**, 127 (1958).
PERLOFF, W. H., JACOBSOHN, G.: Effect of HCG on urinary 17-ketosteroids in a patient with Stein-Leventhal syndrome before and after oophorectomy. J. clin. Endocr. **23**, 1177 (1963).
PLATE, W. P.: The pathologic anatomy of the Stein-Leventhal syndrome. Fertil. Steril. **9**, 545 (1958).
PRUNTY, F. T. G.: Hirsutism, virilism and apparent virilism and their gonadal relationship, part II. J. Endocr. **38**, 85, 203 (1967).
RICE, B. F., HAMMERSTEIN, J., SAVARD, K.: Steroid hormone formation in the human ovary. III. Action of gonadotropins on testosterone synthesis by normal ovarian stromal tissue. Steroids **4**, 199 (1964).
RICKS, PH., KIMINETZKY, H. A., SWERDLOW, M.: Variable response to treatment of the polycystic ovary. Amer. J. Obstet. Gynec. **97**, 64 (1967).
RIONDEL, A., TAIT, J. F., GUT, M., TAIT, S. A. S., JOACHIM, E., LITTLE, B.: Estimation of testosterone in human peripheral blood using S 35-thiosemi-carbazide. J. clin. Endocr. **23**, 620 (1963).
ROBERTS, D. W., HAINES, M.: Is there a Stein-Leventhal syndrome? Brit. med. J. **1960 I**, 1709.
RYAN, K. J., SMITH, O. W.: Biogenesis of steroid hormones in the human ovary. Recent Progr. Hormone Res. **21**, 367 (1965).
SAVARD, K., MARSH, J. M., RICE, B. F.: Gonadotropins and ovarian steroidogenesis. Recent Progr. Hormone Res. **21**, 285 (1965).
SCOTT, R. B.: Thickening of the ovarian tunica; effects of its production artificially upon ovulation in monkeys. Fertil. Steril. **7**, 44 (1956).
SCULLY, R. E.: Androgenic lesions of the ovary. In: The ovary. H. G. GRADY, D. E. SMITH, Eds., p. 143. Baltimore: Williams & Wilkins 1963.
SHEARMAN, R. P., COX, R. I.: The enigmatic polycystic ovary. Obstet. gynec. Surv. **21**, 1 (1966).
— — GANNON, A.: Urinary pregnanetriolone in the diagnosis of Stein-Leventhal syndrome. Lancet **1961 I**, 260.
SHIPPEL, S.: The ovarian theca cell IV. The hyperthecosis syndrome. J. Obstet. Gynaec. Brit. Emp. **62**, 321 (1955).

SHORT, R.V.: Steroids concentrations in the fluid from normal and polycystic (Stein-Leventhal) ovaries. In: Proc. 2. Int. Congr. Endocrin. London 1964 Excerpta med. Found., Amsterdam 1965.
— LONDON, D.R.: Defective synthesis of ovarian steroids in the Stein-Leventhal syndrome. Brit. med. J. **1961 I**, 1724.
SMITH, K.D., STEINBERGER, L., PERLOFF, W.H.: Polycystic ovarian disease. Amer. J. Obstet. Gynec. **93**, 994 (1965).
SOHVAL, A.R., SOFFER, L.: The influence of cortisone and adrenocorticotropin on urinary gonadotropin excretion. J. clin. Endocr. **11**, 677 (1951).
SOMMERS, S.C., WADMAN, P.J.: Pathogenesis of polycystic ovaries. Amer. J. Obstet. Gynec. **72**, 160 (1956).
STAEMMLER, H.J.: Die gestörte Regelung der Ovarialfunktion, p. 220. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
STEIN, I.F.: Duration of fertility following ovarian wedge resection: Stein-Leventhal syndrome. West. J. Surg. **72**, 237 (1964).
— In Discussion. Amer. J. Obstet. Gynec. **97**, 97 (1967).
— LEVENTHAL, M.L.: Amenorrhea associated with bilateral polycystic ovaries. Amer. J. Obstet. Gynec. **29**, 181 (1935).
STERN, M.I., BARWELL, J.O.H.: The measurement of urinary pregn-5-ene-3β,17α,-20α-triol with particular reference to the Stein-Leventhal syndrome. J. Endocr. **27**, 87 (1963).
TAYMOR, M.L., BARNARD, R.: Luteinizing hormone excretion in the polycystic ovary syndrome. Fertil. Steril. **13**, 501 (1962).
— CLARK, B.J., STURGIS, S.H.: The polycystic ovary; a clinical and laboratory study. Amer. J. Obstet. Gynec. **86**, 188 (1963).
VARA, P., NIEMINEVA, K.: Small cystic degeneration of ovaries as incidental finding in gynecological laparatomies. Acta obstet. gynec. scand. **31**, 94 (1951).
WARREN, J.C., SALHANICK, A.A.: Steroid biosynthesis in the human ovary. J. clin. Endocr. **21**, 1218 (1261).
WIELE, R.L. VAN DE, TURKSOY, R.N.: Treatment of amenorrhea and of anovulation with human menopausal and chorionic gonadotropins. J. clin. Endocr. **25**, 369 (1965).
WILSON, H., LIPSETT, M.B., RYAN, D.W.: Urinary excretion of Δ^5-pregnenetriol and other 3β-hydroxy-Δ5 steroids by subjects with and without endocrine disease. J. clin. Endocr. **21**, 1304 (1961).
ZANDER, J., WIEST, W.G., OBER, K.G.: Klinische, histologische und biochemische Beobachtungen bei polycystischen Ovarien mit gleichzeitiger adenomatöser, atypischer Hyperplasie des Endometriums. Umwandlung von Progesteron-4-C 14 im Ovarialgewebe. Arch. Gynäk. **196**, 481 (1962).
ZENER, F.B.: Adrenal androgenic hyperfunction and infertility. Fertil. Steril. **12**, 25 (1961).

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APPLEBY, B.P.: A study of premenstrual tension in general practice. Brit. med. J. **1960 I**, 391.
ARTNER, J.: Das praemenstruelle Syndrom. Zbl. Gynäk. **87**, 145 (1965).
BARFIELD, W.E., JUNGCK, E.C., GREENBLATT, R.B.: The premenstrual tension syndrome: A comprehensive approach to treatment with a progestin-diuretic-tranquilizer combination. Sth. med. J. (Bgham, Ala.) **55**, 1139 (1962).
BICKERS, W.: Uterine contractions in dysmenorrhoe. Amer. J. Obstet. Gynec. **43**, 1023 (1941).
— Premenstrual tension and its relationship to water metabolism. Amer. J. Obstet. Gynec. **64**, 587 (1952).
BLEULER, M.: Endokrinologische Psychiatrie. Stuttgart: Thieme 1954.
BRUCE, J., RUSSELL, F.M.: Premenstrual tension: Study of weight changes and balances of water, sodium and potassium. Lancet **1962 II**, 267.
CIMBERLE, E.: Primi rilieri dell, aldosteronuria nella sindroma premestruale. Minerva ginec. **13**, 997 (1961).
CLITHERTOE, H.J., PICKLES, V.R.: The separation of the smooth muscle stimulants in menstrual fluid. J. Physiol. (Lond.) **156**, 225 (1961).
CONDRAU, G.: Psychosomatik der Frauenheilkunde. Berne: Huber 1965.
COTTE, G.: Resection of presacral nerve in treatment of obstinate dysmenorrhea. Amer. J. Obstet. Gynec. **33**, 1034 (1937).
DALTON, K.: Similarity of symptomatology of premenstrual syndrome and toxaemia of pregnancy and their response to progesterone. Brit. med. J. **1954 II**, 1071.
DAVIS, A.A.: Dysmenorrhea: its aetiology, pathology and treatment. London and Oxford: University Press 1938.
DEUTSCH, H.: Psychologie der Frau, vols. I and II. Berne, Stuttgart: Huber 1953, 1959.
DORMAT, A., HENOCQ, E.: Syndrome prémenstruel et infection génitale. Presse méd. **65**, 2077 (1957).
DOSTER, M.E., MCNIFF, A.L., LAUPE, J.M., CORLISS, L.M.: A survey of menstrual function among 1668 secondary school girls and 720 women employees of the Denver public schools. Amer. J. publ. Hlth **51**, 1841 (1961).
EGLINTON, G., HALL, W.J., PICKLES, V.R., RAPHAEL, R.A., SMITH, G.N.: Isolation and identification of two smooth muscle stimulants from menstrual fluid. Nature (Lond.) **200**, 960 (1963).
EICHNER, E.: Premenstrual tension syndrome and psychogenic dysmenorrhea. Ohio St. med. J. **55**, 953 (1959).
FLUHMANN, C.F.: Endocrine theories of dysmenorrhea. Endocrinology **23**, 393 (1938).
— The management of menstrual disorders. Philadelphia: Saunders 1956.
— Dysmenorrhea. Clin. Obstet. Gynec. **6**, 718 (1963).
FRANK, R.T.: The hormonal cause of premenstrual tension. Arch. Neurol. Psychiat. (Chic.) **26**, 1053 (1931).
FREED, S.C.: The treatment of premenstrual distress. J. Amer. med. Ass. **127**, 372 (1945).
GALLAGHER, J.R.: Medical care of the adolescent. New York: Appleton-Century-Crofts 1960.
GOLUB, L.J., MENDUKE, H., LANG, W.R.: One component of an exercise for dysmenorrhea. Obstet. and Gynec. **22**, 324 (1963).
GREENBLATT, R.B., HAMMOND, D.O., CLARK, S.L.: Membranous dysmenorrhea: studies and etiology and treatment. Amer. J. Obstet. Gynec. **68**, 835 (1954).
GREENE, R., DALTON, K.: The premenstrual syndrome. Brit. med. J. **1953 I**, 1007.
GREENHILL, J.P., FREED, S.C.: The electrolytic therapy of premenstrual tension. J. Amer. med. Ass. **117**, 504 (1941).
HAMAN, J.O.: Pain threshold in dysmenorrhea. Amer. J. Obstet. Gynec. **47**, 686 (1944).
HAUSER, G.A., MARTI, M., WENNER, R.: Das praemenstruelle Syndrom. Geburtsh. Frauenheilk. **19**, 299 (1959).
HEALD, F.P., Jr., MASLAND, R.P., Jr., STURGIS, S.H., GALLAGHER, J.R.: Dysmenorrhea in adolescence. Pediatrics **20**, 121 (1957).
HELD, E.: Die erweiterte hypogastrische Sympathektomie zur operativen Bekämpfung schwerer Dysmenorrhoen. Zbl. Gynäk. **67**, 245 (1943).
HERSCHBERG, A.D.: Premiers essais de psychoprophylaxie de la dysmenorrhée primaire fonctionelle. C. R. Soc. franç. Gynec. **30**, 357 (1960).
ISRAEL, S.L.: Premenstrual tension. In: Menstrual disorders and sterility, ISRAEL, S.L., Ed., p. 152. New York: Harper & Row 1967.
JAMES, M.: Hysterography as an aid in some problems posed by dysmenorrhea: II. Anatomic and functional isthmic factors. Gynéc. et Obstét. **62**, 679 (1963).
JEFFCOATE, T.N.A., LERER, S.: Hypoplasia of the uterus with special reference to spasmodic dysmenorrhea. J. Obstet. Gynaec. Brit. Emp. **52**, 97 (1945).

JENKINS, J.S.: Progesterone and norethisterone in cyclical oedema and ascites. Brit. med. J. **1961II**, 861.
KISTNER, R.W.: Gynecology. Principles and practice. Year book Medical Publ., Chicago, 1964.
KÖNIG, P.A.: Statistischer Beitrag zum Dysmenorrhoe-Problem. Arch. Gynäk. **192**, 174 (1959).
KRETSCHNER, W., Jr.: Die Neurose als Reifungsproblem. Stuttgart: Thieme 1952.
KROGER, W.S.: Psychosomatic obstetrics, gynecology and endocrinology. Springfield: Thomas 1962.
LAMB, W.M., ULETT, G.A., MASTERS, W.H., ROBINSON, D.W.: Premenstrual tension: EEG: Hormonal and psychiatric evaluation. Amer. J. Psychiat. **109**, 840 (1953).
LAMSON, E.T., EDMADJIAN, F., HOPE, J.M., PINCUS, G., JORJORIAN, D.: Aldosterone excretion of normal schizophrenic and psychoneurotic subjects. J. clin. Endocr. **16**, 954 (1956).
LANDAU, R.L., LUGIBIHL, A.: Inhibition of the sodium retaining influence of aldosterone by progesterone. J. clin. Endocr. **18**, 1237 (1958).
— — The catabolic and natriuretic effects of progesterone in man. Recent Progr. Hormone Res. **17**, 249 (1961).
LOMER, K.: Zur Beurteilung des Schmerzes in der Gynäkologie. Wiesbaden: Bergmann 1899.
LOWE, C.R., FERGUSON, R.C.: Incidence of dysmenorrhea. Brit. J. soc. Med. **5**, 193 (1951).
LUKAS, K.H.: Die Dysmenorrhoe. Habilitationsschrift Tübingen, 1963.
— Die Dysmenorrhoe. Beilageheft zu Z. Geburtsh. Gynäk. **163**, 1 (1965).
— Primäre Dysmenorrhoe und Neurose. Z. Geburtsh. Gynäk. **147**, 339 (1956).
MACHT, D.I.: Concerning the chemical nature of menstrual toxin. Amer. J. Obstet. Gynec. **57**, 251 (1949).
MANN, E.C., MCLARN, W.D., HAYT, D.B.: The physiology and clinical significance of the uterine isthmus. Amer. J. Obstet. Gynec. **81**, 209 (1961).
MAYER, A.: Über Reifungsprobleme in der Fortpflanzungsepoche der Frau in gynäkologischer Sicht. In: Mehrdimensionale Diagnostik und Therapie. Stuttgart: Thieme 1958.
MCKINNON, P.C.B., MCKINNON, J.L.: Hazards of menstrual cycle. Brit. med. J. **1956I**, 555.
MENZER-BENARON, D., MORRIS, T.A., SABBATH, J., STURGIS, S.H.: Premenstrual tension. In: The gynecological patient, STURGIS, S.H., Ed. New York: Grune & Stratton 1963.
MICHEL-WOLFROMM, H.: Gynécologie psychosomatique. Paris: Masson 1963.
MILLER, N.F.S., BEHRMAN, J.: Dysmenorrhea. Amer. J. Obstet. Gynec. **65**, 505 (1953).
MOIR, J.C.: Discussion on intrinsic dysmenorrhea. Proc. roy. Soc. Med. **29**, 950 (1936).
MORTON, J.H., ADDISON, H., ADDISON, R.G., HUNT, L., SULLIVAN, J.J.: A clinical study of prementrual tension. Amer. J. Obstet. Gynec. **65**, 1182 (1953).
PRILL, H.J.: Gynäkologie. In: Handbuch der Neurosenlehre und Psychotherapie, FRANKL, V.E., V.E. VON GEBSATTEL, J.H. SCHULTZ, Eds., Bd. V, Grenzgebiete und Grenzfragen. Munich: Urban & Schwarzenberg 1961.
— Zur Frage der Ischaemie bei Dysmenorrhoe. Geburtsh. Frauenheilk. **22**, 1018 (1962).
— Psychosomatische Gynäkologie. Munich: Urban & Schwarzenberg 1964.
— KRÜGER, E.: Uber die Oestrogenausscheidung bei Frauen mit praemenstruellem Syndrom. Endokrinologie **44**, 34 (1963).
REES, L.: Psychosomatic aspects of the premenstrual tension syndrome. J. ment. Sci. **99**, 62 (1953).
RHEINGOLD, J.C.: The fear of being a woman. New York: Grune & Stratton 1964.
ROGERS, J.: Endocrine and metabolic aspects of gynecology. Philadelphia: Saunders 1963.
RÖMER, H.: Gynäkologische Organneurosen. Stuttgart: Thieme 1953.
— Das Sexualleben der Frau und seine Störungen. In: Gynäkologie und Geburtshilfe, KÄSER, O., Ed., p. 487. Stuttgart: Thieme 1969.
SMITH, O.W., SMITH, G. VAN S.: Menstrual toxin. In: Menstruation and its disorders, ENGLE, E.T., Ed. Springfield: Thomas 1950.
STREETEN, D.H.P.: The use of aldosterone antagonists in idiopathic edema. In: The clinical use of aldosterone antagonists, BARTTER, F.C., Ed. Springfield: Thomas 1960.
STURGIS, S.H.: The gynecologic patient. New York: Grune & Stratton 1962.
THORN, G.W.: Cyclical edema. Amer. J. Med. **23**, 507 (1957).
— NELSON, K.R., THORN, D.W.: A study of the mechanism of edema associated with menstruation. Endocrinology **22**, 155 (1938).
VENNING, E., DYRENFURTH, J., GIROUD, C.J.P.: Aldosterone excretion in healthy persons. J. clin. Endocr. **16**, 1326 (1957).
WALTHARD, M.: Die Beziehungen des Nervensystems zu den normalen Betriebsabläufen und zu den funktionellen Störungen im weiblichen Genitale. In: Handbuch der Gynäkologie, 3rd ed., Bd. XI, STOECKEL, W., Ed. Munich: Bergmann 1937.
WILLIAMS, E.Y., WEEKES, L.R.: Premenstrual tension associated with psychotic episodes. J. nerv. ment. Dis. **116**, 321 (1952).
WILSON, L., KURZROK, R.: Uterine-contractility in functional dysmenorrhea. Endocrinology **27**, 23 (1940).
WOODBURY, R.A., TORPIN, R., CHILD, R., WATSON, H., JARBOE, M.: Myome trial physiology and its relation to pelvic pain. J. Amer. med. Ass. **314**, 1081 (1947).
YOUSSEF, A.F.: The uterine isthmus and its sphincter mechanism (a radiographic study). I. The uterine isthmus under normal conditions. II. The uterine isthmus under abnormal conditions. Amer. J. Obstet. Gynec. **75**, 1305, 1320 (1958).
ZUSPAN, F.P., BARNES, A.C., DILLHOFER, J.R.: The urinary excretion of antidiuretic substances by the obstetric and gynecologic patient. Amer. J. Obstet. Gynec. **76**, 619 (1958).

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ABARBANEL, A.R.: Transvaginal pelvioscopy. Further studies in infertility. Proc. 2nd World Congr. Fertil. Steril. (Naples, 1956) **1**, 1140 (1958).
ANTOINE, T.: Die Bedeutung des Cervixfaktors für die Sterilität. Arch. Gynäk. **189**, 245 (1957).
BALIN, H.: Sterility: General considerations. In: Diagnosis and treatment of menstrual disorders and sterility, S.L. ISRAEL, Ed., 5th ed. New York: Hoeber 1967.
BARNS, T., CAMPBELL, H., SNAITH, L.: The failure of therapy in the management of infertility. J. Obstet. Gynaec. Brit. Emp. **60**, 670 (1953).
BARTON, M., WIESNER, B.P.: Waking temperature in relation to female fecundity. Lancet **1945II**, 663.
BEHRMAN, S.J.: Agglutinins, antibodies and immune reactions. Clin. Obstet. Gynec. **8**, 91 (1965).
— BÜTTNER-JANUSCH, J., HEGLAR, R., GERSKOWITZ, H.: ABO (H) blood incompatibility as a cause of infertility. Amer. J. Obstet. Gynec. **79**, 847 (1960).
BERNHARD, P.: Die Sterilität des Weibes. Stuttgart: Enke 1947.
BETTENDORF, G.: Wirkungen und klinische Anwendung von Clomiphen. 68. Tagung Nordwestd. Ges. Gynäk., Göttingen 1967.
BICKENBACH, W., DÖRING, G.K.: Die Sterilität der Frau, 3rd ed. Stuttgart: Thieme 1967.
BROWN, A.B., CHAMBERLAIN, R., TELINDE, R.W.: Myomectomy. Amer. J. Obstet. Gynec. **71**, 759 (1956).

BROWN, W. E., BRADBURY, J. T.: A study of the physiologic action of human chorionic hormone. Amer. J. Obstet. Gynec. **53**, 749 (1947).

BUXTON, C. L., SOUTHAM, A. L.: Human infertility. New York: Hoeber 1958.

CABALLERO, A.: Epidemiology of tuberculosis of the female genital tract. In: Latent female genital tuberculosis, RIPPMANN, E. T., R. WENNER, Eds., p. 36. Basle, New York: Karger 1966.

CAMPOS DA PAZ, A.: The cristallizationtest as a guide to the treatment of cervical hostility. Fertil. Steril. **4**, 137 (1953).

CHANG, M. C.: Development of fertilizing capacity of rabbit spermatozoa in the uterus. Nature (Lond.) **175**, 1036 (1955).

COHEN, M. R., KAYE, B. M.: Spinnbarkeit: A characteristic of cervical mucus. Significance at ovulation time. Fertil. Steril. **3**, 201 (1952).

COLLETT, M. E., WERTENBERGER, G. E., FIS SE, V. M.: The effect of age upon the pattern of the menstrual cycle. Fertil. Steril. **5**, 437 (1954).

CROOKE, A. C.: Gonadotropin sensitivity test. Acta endocr. (Kbh.), Suppl. **100**, 109 (1965).

DANEZIS, J. M.: Studi sulla sterilità casa editrice Pietro Cairoti. Como 1955, cited by STALLWORTHY, J. A., G. L. BOURNE: Recent advances in obstet. and gynaec. London: Churchill 1966.

DAVIDS, A. M.: The management of fibromyomas in infertility and abortion. Clin. Obstet. Gynec. **2**, 837 (1959).

DAVIDSON, H. A.: Med. Press **245**, 18 (1961); cited by STALLWORTHY, J., G. BOURNE: Recent advances in obstet. gynaec., p. 286. London: Churchill 1966.

DIDDLE, H. F., JACK, R. W., PERSE, R. L.: Fertility in women: The length of time to conceive. Amer. J. Obstet. Gynec. **54**, 57 (1947).

DÖRING, G. K.: Der Temperaturzyklus der Frau. Ärztl. Forsch. **6**, 13 (1952).

— Über die Behandlung der Sterilität bei Gelbkörperschwäche. Z. Geburtsh. Gynäk. (1960), Suppl., p. 95.

— Über die relative Häufigkeit des anovulatorischen Zyklus im Leben der Frau. Arch. Gynäk. **199**, 115 (1963).

— BICKENBACH, W.: Die Bestimmung der fruchtbaren und unfruchtbaren Tage der Frau mit Hilfe der Körpertemperatur. Stuttgart: Thieme 1957.

DOYLE, J. B.: Exploratory culdotomy for observation of tuboovarian physiology at ovulation time. Fertil. Steril. **2**, 475 (1951).

DUNCAN, J. M.: Fecundity, fertility, sterility and allied topics. Edinburgh: A. & C. Black 1866.

ELERT, R.: Die Berücksichtigung der zyklusabhängigen Zervixschleimveränderungen bei den Penetrationstesten. Deutsche u. Österr. Ges. z. Stud. Sterilität, Salzburg 1963.

FISHER, K. H.: Tuberculous endometritis diagnosed 12 days after abortion. Brit. med. J. **1962 II**, 711.

FRANGENHEIM, H.: Die Laparoskopie und die Culdoskopie in der Gynäkologie. Stuttgart: Thieme 1959.

FUMASOLI, H. J.: Ergebnisse der Prednisonbehandlung bei Adnexitis. Dissertation, Zurich 1970.

GILES, A. E.: Menstruation and its disorders. London: Baillière, Tyndall 1901.

GILLAM, J. S.: Study of the inadequate secretion phase endometrium. Fertil. Steril. **4**, 18 (1955).

GRANT, A.: Cervical hostility; incidence, diagnosis and prognosis. Fertil. Steril. **9**, 321 (1958).

— An evaluation of the conservative treatment of endometriosis. Aust. N.Z. J. Obstet. Gynaec. **3**, 162 (1963).

— The diagnosis and treatment of endometriosis in young women. Int. J. Fertil. **9**, 503 (1964).

— MCBRIDE, W. G., NOYES, J. M.: Luteal phase defects in sterility. Int. J. Fertil. **4**, 315 (1959).

GREEN, TH. H.: Conservative surgical treatment of endometriosis. Clin. Obstet. Gynec. **9**, 293 (1966).

GREENBLATT, R. B.: Chemical induction of ovulation. Fertil. Steril. **12**, 402 (1961).

GREENHILL, J. P.: Present studies of plastic operations on the fallopian tubes. Amer. J. Obstet. Gynec. **72**, 516 (1956).

GRUBER, M.: Untersuchungsresultate bei 106 Fällen mit primärer Sterilität. Dissertation, Zurich 1973.

GUERRERO, C. D.: Management of the infertile couple: 25 years experience. Fertil. Steril. **15**, 534 (1964).

HAGERMAN, D. D. O., SMITH, O. W., DAY, C. F.: Mechanism of stimulatory effect of Clomid on aromatization of steroids by human placenta *in vitro*. Acta endocr. (Kbh.) **51**, 591 (1966).

HAMBLEN, E. C.: Facts for childless couples. Springfield: Thomas 1942.

HARTMAN, C. G.: Science and the safe period. A compendium of human reproduction. Baltimore: Williams & Wilkins Comp. 1962.

HAUSER, G. A., SCHREINER, W. E.: Das Mayer-Rokitansky-Küster-Syndrom. Schweiz. med. Wschr. **61**, 381 (1961).

HELD, E.: Les contractions tubaires et leur réaction à la pitocine au cours du cycle menstruel. Helv. med. Acta **7**, 45 (1940).

— Das Ergebnis des Probestrichs bei sterilen Frauen mit durchgängigen Tuben. Lugano, 1945. Mtschr. Geburtsh. Gynäk. **119**, 271 (1945).

— Die weibliche Genitaltuberkulose. Gynaecologia (Basel) **123**, 265 (1947).

HERTIG, A. T., ROCK, J., ADAMS, E.: A description of 34 human ova within the first 17 days of development. Amer. J. Anat. **98**, 435 (1956).

HOTCHKISS, R. S.: Fertility in men. Philadelphia-Montreal-London: Lippincott 1944.

HUHNER, M.: Sterility in the male and female and its treatment. New York: Rebmann Co 1913.

HUSSLEIN, H.: Zervixschleim und Sterilität. Zbl. Gynäk. **75**, 1574 (1953).

ISRAEL, S. L.: Treatment of menstrual disorders and sterility. New York: Hoeber, P. B. 1967.

JAVERT, C. T.: Spontaneous and habitual abortion. New York-Toronto-London: McGraw-Hill 1957.

JAYLE, M. F., GUEGUEN, J., VALLIN, Y., VEYRIN-FORRER, F.: Exploration dynamique de la fonction lutéale. Presse méd. **64**, 1956 (1965).

JEFFCOATE, T. N. A.: Proc. Soc. Study Fertil. **5**, 1 (1953); cited by STALLWORTHY, J., G. BOURNE, Recent advanc. obstet. gynaec., p. 286. London: Churchill 1966.

JOHNSON, J. E.: Outcome of pregnancies following clomiphene citrate therapie. Presented at 5th world congress on fertility and sterility, Stockholm 1966.

KATSH, S.: Immunologic aspects of infertility and conception control. In: Advances in Obstet. Gynec., MARCUS, S. L., MARCUS, C. C., Eds. Baltimore: The Williams & Wilkins Comp. 1967.

KELLER, P. J.: Ovulationsauslösung bei Zyklus-Störungen. Schweiz. Z. Gynäk. Geburtsh. (in press).

— NAVILLE, A. H., WYSS, H. J.: Medikamentöse Beeinflussung der hypophysären Gonadotropine durch Clomiphen. Gynaecologia (Basel) **162**, 402 (1966).

KELLY, J. V., ROCK, J.: Culdoscopy for diagnosis in infertility: Report of 492 cases. Amer. J. Obstet. Gynec. **72**, 523 (1956).

KISTNER, R. W.: Infertility with endometriosis. Fertil. Steril. **13**, 237 (1962).

— Gynecology, principles and practice. Chicago: Year Book Medical Publ. Inc. 1964.

— Induction of ovulation with clomiphene citrate (Clomid). Obstet. gynec. Surv. **20**, 873 (1965).

— Current status of the hormonal treatment of endometriosis. Clin. Obstet. Gynec. **9**, 271 (1966).

KOPPEN, K.: Die Abhängigkeit der Fruchtbarkeit der Frau von ihrem Alter. Z. Geburtsh. Gynäk. **134**, 237 (1951).

MAGGIONI, G.: Studi sulla sterilità. Casa editrice PIETRO CAIROLI. Cited by STALLWORTHY, J. A., G. L. BOURNE, Recent advances in Obstet. Gynaec. London: Churchill 1966.

MARCUS, C.C., MARCUS, ST.L.: Advances in infertility. In: Advances Obstet. Gynec., vol. I, p. 439, MARCUS, ST.L., C.C. MARCUS, Eds. Baltimore: Williams & Wilkins 1967.

MARTIUS, H.: Lehrbuch der Gynäkologie, 8th ed. Stuttgart: Thieme 1964.

MASTERS, W.H., JOHNSON, V.E.: Intravaginal environment. I. A lethal factor. Fertil. Steril. **12**, 560 (1961).

MCARTHUR, J.W., ULFELDER, H.: Effects of pregnancy upon endometriosis. Obstet. Gynec. Surv. **20**, 709 (1965).

MCCOY, J.B., BRADFORD, W.Z.: Surgical treatment of endometriosis with conservation of reproductive potential. Amer. J. Obstet. Gynec. **87**, 392 (1963).

MEAKER, S.R.: Male infertility from a gynecologic viewpoint. J. Urol. (Baltimore) **43**, 871 (1940).

MILLER, E.G., KURZROK, R.: Biochemical studies of human semen: III. Factors affecting migration of sperm through the cervix. Amer. J. Obstet. Gynec. **24**, 19 (1932).

MROUEH, A., GLASS, R.H., BUXTON, C.L.: Tubal plastic surgery. Fertil. Steril. **18**, 80 (1967).

MÜNZNER, H., LÖER, K.: Statistische Untersuchungen über die Abhängigkeit der weiblichen Fruchtbarkeit vom Alter. Zbl. Gynäk. **58**, 2900 (1934).

NOTTEBOHM, L.: Über die Wahrscheinlichkeit der Konzeption innerhalb eines Zyklus bei vorhandenem Kinderwillen. Inaugural dissertation, Göttingen 1948.

OBER, K.G.: Aufwachtemperatur und Ovarialfunktion. Klin. Wschr. **30**, 357 (1952).

PALMER, R.: Diagnostic de la valeur et de la ducrée du corps jaune menstruel. Rev. franç. gynéc. **44** (1949).

— La stérilité involontaire. Paris: Masson 1950.

— Les résultats du traitement chirurgical des obturations tubaires. Féd. Soc. Gynéc. Obstét. franç. **4**, 497 (1952).

— La stérilité par insuffisance lutéale et son traitement par la gonadotropine chorionique. Ann. Obstet. Gynec. **75**, 951 (1953).

— Les explorations fonctionelles gynécologiques dans la stérilité, l'avortement récidivant et les troubles menstruels. Paris: Masson 1963.

PAPANICOLAOU, G.N.: General survey of a vaginal smear and its use in research and diagnosis. Amer. J. Obstet. Gynec. **51**, 316 (1946).

PARISH, W.E., WARD, A.: Studies of cervical mucus and serum from infertile women. J. Obstet. Gynaec. Brit. Cwlth **75**, 1089 (1968).

PEARL, R.: The natural history of population. New York: Oxford Univ. Press 1939.

PEEL, J.H.: The role of major surgery in infertility. Amer. J. Obstet. Gynec. **71**, 706 (1956).

PERETZ, A., SHARF, M.: Culdoscopy in gynecologic diagnosis. Amer. J. Obstet. Gynec. **82**, 582 (1961).

PHILIPP, E.: Zur Klinik der Endometriosen. Zbl. Gynäk. **61**, 2 (1937).

PLOTZ, J.: Der Wert der Basaltemperatur für die Diagnose der Menstruationsstörungen. Arch. Gynäk. **177**, 521 (1950).

PUCK, A.: Der Einfluß des Oestriols auf das Sekret der Cervix uteri. Geburtsh. Frauenheilk. **18**, 998

RAUSCHER, H., LEEB, H.: Zur Diagnostik und Therapie bei Insuffizienz der Gelbkörperwirkung bei Frauen mit Kinderwunsch. Wien. med. Wschr. **109**, 279 (1959).

RIBY, G.M., DONTAS, E., GILL, B.: Use of serial vaginal smears in detecting the time of ovulation. Fertil. Steril. **6**, 86 (1955).

RIPPMANN, E.T., WENNER, R.: Latent female genital tuberculosis. Basel, New York: Karger 1966.

RIVA, H.L., ANDRESON, P.S., ROSIERS, J.L. DES, BREEN, J.L.: Further experience with culdoscopy. J. Amer. med. Ass. **178**, 873 (1961).

— HATCH, R.P., BREEN, J.L.: Culdoscopy for infertility: analysis of 203 cases. Amer. J. Obstet. Gynec. **78**, 1304 (1959).

— KAWASAKI, D.M., MESSINGER, A.J.: Further experience with norethynodrel in treatment of endometriosis. Obstet. and Gynec. **19**, 111 (1962).

— WILSON, J.H., KAWASAKI, D.M.: Effect of norethynodrel on endometriosis. Amer. J. Obstet. Gynec. **82**, 109 (1961).

ROCK, J.: Physiology of human conception. New Engl. J. Med. **240**, 804 (1949).

ROCKER, I.: The anatomy of the uterotubal junction area. Proc. roy. Soc. Med. **57**, 707 (1964).

ROEMER, H.: Das Sexualleben der Frau und seine Störungen. In: Gynäkologie und Geburtshilfe, KÄSER, O., Ed., Bd. I, p. 487. Stuttgart: Thieme 1967.

ROY, S.R., GREENBLATT, R.B., MAHESH, V.B., JUNGCK, E.C.: Clomiphene citrate: Further observations on its use in induction of ovulation in the human and on its mode of action. Fertil. Steril. **14**, 575 (1963).

— MAHESH, V.B., GREENBLATT, R.B.: Effects of clomiphene on the physiology of reproduction in the rat. III. Inhibition of uptake of radioactive oestradiol by the uterus and the pituitary gland of immature rat. Acta endocr. (Kbh.) **47** (1964).

RUBENSTEIN, B.B.: Estimation of ovarian activity by the consecutive-day study of basal body temperature and basal metabolic rate. Endocrinology **22**, 41 (1938).

RUBIN, I.C.: The nonoperative determination of patency of fallopian tubes. J. Amer. med. Ass. **75**, 661 (1920).

— Twelve years experience with uterotubal insufflation; diagnostic and therapeutic. Amer. J. Obstet. Gynec. **24**, 561 (1932).

— Uterotubal insufflation. St. Louis: C.V. Mosby Comp. 1947.

RYDBERG, E.: Observation on the crystallization of the cervical mucus. Acta obstet. gynec. scand. **28**, 172 (1949).

RYDEN, A.: Acta obstet. gynec. scand. **37**, 114 (1958); cited in STALLWORTHY, J., G. BOURNE, Rec. advances in obstet. gynaec., p. 286. London: Churchill 1966.

SCHAEFER, G.: Full-term pregnancy following genital tuberculosis. Obstet. Gynec. Surv. **19**, 81 (1964).

SCHILDBACH, H.R., DAHN, H.: Beobachtungen und Ergebnisse bei Hysterosalpingographien und Sterilitätsbehandlungen. Arch. Gynäk. **177**, 302 (1950).

SCHREINER, W.E.: Die Auslösung der Ovulation mit Clomiphen. Dtsch. med. Wschr. **92**, 1473 (1967).

SÉGUY, J., VIMEUX, J.: Contribution à l'étude des stérilités inexpliquées: étude de l'ascension des spermatozoides dans les voies basses de la femme. Gynéc. Obstét. **27**, 346 (1933).

SEMM, K.: Prüfung der Tubendurchgängigkeit. In: Gynäkologie und Geburtshilfe, KÄSER, O., Bd. I, p. 857. Stuttgart: Thieme 1969.

SHARMAN, A.: Endometrial tuberculosis in sterility. Fertil. Steril. **3**, 144 (1952).

— Some lessons from 4000 uterotubal insufflations. Brit. med. J. **1954I**, 239.

SIEGLER, M., HELLMAN, L.M.: Tubal plastic surgery. Fertil. Steril. **7**, 170 (1956).

SIMMONS, F.A.: The diagnosis and treatment of the infertile female. Springfield/Ill.: Ch.C. Thomas, Publ. 1954.

SIMS, J.M.: On the microscope as an aid in the diagnosis and treatment of sterility. N.Y. med. J. **8**, 393 (1869).

SLOAN, W.R.: Infertility in northern Ireland. J. Obstet. Gynaec. Brit. Cwlth **71**, 404 (1964).

SNAITH, L.M., BARNS, T.: Fertility in pelvic tuberculosis. Lancet **1962I**, 712.

SOBRERO, A.J.: Bacteriological findings in the midcycle endocervical mucus in infertile women. Ann. N.Y. Acad. Sci. **97**, 591 (1962).

SOUTHAM, A.: What to do with the normal infertile couple? Fertil. Steril. **11**, 543 (1960).

STALLWORTHY, J.: Fertility and genital tuberculosis. Fertil. Steril. **14**, 284 (1963).

STALLWORTHY, J.A., BOURNE, G.L.: Fertility and sterility. In: Recent advances in Obstet. Gynaec. London: J. & A. Churchill, Ltd. 1966.

STAMER, S.: Acta obstet. gynec. scand. **26**, 263 (1946); cited by STALLWORTHY, J., G. BOURNE, Rec. advanc. in Obstet. Gynaec., p. 286. London: Churchill 1966.
STEVENSON, C.S.: Myomectomy for improvement of fertility. Fertil. Steril. **15**, 367 (1964).
STOECKEL, W.: Lehrbuch der Gynäkologie, 14th ed. Leipzig: Hirzel 1960.
SUTHERLAND, A.M.: The results of treatment of genital tuberculosis in women. In: Latent female genital tuberculosis, RIPPMANN, E.T., R. WENNER, Eds., p. 196. Basel, New York: S. Karger 1966.
SWEENEY, W.J.: Pitfalls in present-day methods of evaluating tubal function. I. Tubal insufflation. Fertil. Steril. **13**, 113 (1962).
TANNER, E., BAER, P., WANNER, J.: Die „Schaukeltherapie" der Lungentuberkulose. Schweiz. med. Wschr. **33**, 751 (1953).
THOMAS, H.H.: Standards for infertility diagnosis. Fertil. Steril. **17**, 779 (1966).
TIETZE, CHR., GUTTMACHER, A.F., RUBIN, S.: Time required for conception in 1727 planned pregnancies. Fertil. Steril. **1**, 338 (1950).
TOMPKINS, P.: The use of basal temperature graphs in determining the date of ovulation. J. Amer. med. Ass. **124**, 698 (1944).
— Basal body temperature graphs as an index to ovulation. J. Obstet. Gynaec. Brit. Emp. **52**, 241 (1945).
TYLER, E.T.: Office management of the infertile couple. New York-Toronto-London: McGraw-Hill 1961.
VELDE, T.H. VAN DE: Über den Zusammenhang zwischen Ovarialfunktion, Wellenbewegung und Menstrualblutung und über die Entstehung des sog. Mittelschmerzes. Haarlem: Bohn 1905.
VOLLMANN, U.: Untersuchungen über die Körpertemperatur der Frau in Korrelation zu den Phasen ihres Genitalzyklus. Mschr. Geburtsh. Gynäk. **111**, 41 (1940).
WESTIN, B., WIGWIST, N.: Fertility and sterility. In: Proceedings of the 5th world congress on Fertil. Steril., Stockholm, 16—22 June, 1966. Excerpta med. (Amst.) Sect. X.
WHITELAW, M.J.: Statistical evaluation of female fertility. Fertil. Steril. **11**, 428 (1960).
WIELE, R.L. VAN DE, TURKSOY, R.N.: Treatment of amenorrhea and of anovulation with human menopausal and chorionic gonadotropin. J. clin. Endocr. **25**, 369 (1965).
WILLIAMS, W.W.: Sterility. The diagnostic survey of the infertile couple. Springfield, Mass.: W.W. Williams 1964.
WYLER, J.: Das Höchstalter der Gebärfähigkeit der Frau. Schweiz. med. Wschr. **87**, 698 (1957).
WYSS, H.I., NAVILLE, A.H., KELLER, P.J.: Die medikamentöse Behandlung der Ovulationsstörungen. Praxis **56**, 332 (1967).

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BARZILAI, G.: Atlas of ovarian tumors. New York: Grune & Stratton 1943.
BAUER, J., KARL, J.: Der chemische und kristalline Nachweis von androgenen und corticoiden Wirkstoffen aus Urin und Geschwulstgeweben bei virilisierendem Ovarialtumor. Z. ges. exp. Med. **118**, 425 (1952).
BAULIEU, E.E., MAUVAIS-JARVIS, P., CORPÉCHAT, C.: Steroid studies in a case of Stein-Leventhal syndrome with hirsutism. J. clin. Endocr. **23**, 374 (1963).
BISKIND, M.S., BISKIND, G.S.: Development of tumors in the rat ovary after transplantation into the spleen. Proc. Soc. exp. Biol. (N.Y.) **55**, 176 (1944).
BRUK, J., DANCASTER, C.P., JACKSON, W.P.U.: Granulosa-cell tumours causing precocius puberty; oestrogen fractionations in two patients. Brit. med. J. **1960 2** 26.
BRYSON, M.J., DOMINGUEZ, O.V., KAISER, I.H., SAMUELS, L.T., SWEAT, M.L.: Enzymatic steroid conversion in a masculinovoblastoma. J. clin. Endocr. **22**, 773 (1962).
BUSBY, T., ANDERSON, G.W.: Feminizing mesenchymomas of the ovary. Includes 107 cases of granulosa-, granulosa-theca-cell-, and theca-cell tumors. Amer. J. Obstet. Gynec. **68**, 1391 (1954).
DEVIS, R.: Cited by MATHET, P. in: Les tumeurs masculinisantes de l'ovaire. Paris Thèse, Libr. Arnette. Paris 1956.
DIDDLE, A.W.: Granulosa- and theca-cell ovarian tumors, prognosis. Cancer (Philad.) **5**, 215 (1952).
DOCKERTY, M.B., MCCARTY, W.C.: Arrhenoblastoma. Surg. Gynec. Obstet. **68**, 767 (1939).
— MUSSEY, E.: Malignant lesions of the uterus associated with estrogen-producing tumors. Amer. J. Obstet. Gynec. **61**, 147 (1951).
EMGE, L.A.: Endometrial cancer and feminizing tumors of the ovaries. Significance of their coexistence. Obstet. Gynec. **1**, 511 (1953).
EMIG, O.R., HERTIG, A.T., ROWE, F.J.: Gynandroblastoma of the ovary: Review and report of a case. Obstet. and Gynec. **13**, 135 (1959).
FLICKINGER, G.L., MURAWEC, T.: Free and conjugated estrogens of an ovarian cystadenoma and granulosa cell tumor. J. clin. Endocr. **25**, 1231 (1965).
GEIST, S.H., GAINES, J.A., POLLACK, A.D.: Experimental biologically active ovarian tumors in mice. Amer. J. Obstet. Gynec. **38**, 786 (1939).
GREENE, R.R., RODDICK, J.W., MILLIGAN, M.: Estrogens, endometrial hyperplasia, and endometrial carcinoma. Ann. N.Y. Acad. Sci. **75**, 586 (1958—1959).
HELD, E., SCHREINER, W.E.: Über einen Fall von Arrhenoblastom. Gynaecologia (Basel) **147**, 402 (1959).
HERTIG, A.T., GORE, H.M.: Tumors of the female sex organs: Part 3. Tumors of the ovary and Fallopian tube. Atlas of tumor pathology, sect. IX, fasc. 33. Washington D.C.: Armed Forces Institute of Pathology 1961.
— — Estrogenic lesions of the ovary. In: The ovary, GRADY, H.G., D.E. SMITH, Eds. Baltimore: Williams & Wilkins Co. 1963.
HODGSON, J.E., DOCKERTY, M.B., MUSSEY, R.D.: Granulosa cell tumor of the ovary. A clinical and pathologic review of sixty-two cases. Surg. Gynec. Obst. **81**, 631 (1945).
HUGHESDON, P.E., FRASER, I.T.: Arrhenoblastoma of ovary: Case report and histological review. Acta obstet. gynec. scand. **32**, Suppl. 4 (1953).
INGRAM, J.M., NOVAK, E.: Endometrial carcinoma associated with feminizing mesenchymal tumors. Amer. J. Obstet. Gynec. **61**, 774 (1951).
JAVERT, C.T., FINN, W.F.: Arrhenoblastoma; the incidence of malignancy and the relationship to pregnancy, to sterility, and to treatment. Cancer (Philad.) **4**, 60 (1951).
JAYLE, M.F.: Données nouvelles sur les fonctions endocriniennes des gonades. Rév. franç. Endocr. clin. **8**, 370 (1967).
JEFFCOATE, S.L., PRUNTY, F.T.G.: Steroid synthesis *in vitro* by a hilar cell tumor. Amer. J. Obstet. Gynec. **101**, 684 (1968).
KASE, N.: Steroid synthesis in abnormal ovaries. Part II. Granulosa cell tumor. Amer. J. Obstet. Gynec. **90**, 1262 (1964).
— CONRAD, S.H.: Steroid synthesis in abnormal ovaries. I. Arrhenoblastoma. Amer. J. Obstet. Gynec. **90**, 1251 (1964).
KESCÉS, L., MUTSCHLER, F., KOBOR, J.: Oestrogenbestimmung im Gewebe bei Tumoren der Ovarien. Zbl. Gynäk. **85**, 325 (1963).
KENT, S.W., MCKAY, D.G.: Primary cancer of the ovary. Amer. J. Obstet. Gynec. **80**, 430 (1960).
KISTNER, R.W.: Gynecology. Year Book Medical Publishers. Chicago 1964.
KÖNIG, F.E.: Granulosa- und Thekazelltumoren und Scheidenabstrich. Gynaecologia (Basel) **135**, 225 (1953).
KOTTMEIER, H.L.: The classification and treatment of ovarian tumours. Acta obstet. gynec. scand. **31**, 313 (1952).

— Carcinoma of the female genitalia. Baltimore: Williams & Wilkins Comp. 1953.
KRAUS, F.T.: Gynecologic pathology. St. Louis: C.V. Mosby, Comp. 1967.
LARSON, J.A.: Estrogens and endometrial carcinoma. Obstet. Gynec. **3**, 551 (1954).
LOBOTSKY, J., LOEWY, B.P., LLOYD, C.W., ROGERS, W.F.: The endocrine Society: 36th meeting, San Francisco June 1954; cited in WILLIAMS, R.H.: Textbook of endocrinology, p. 380. Philadelphia: W.B. Saunders Comp. 1955.
LOEFFLER, E., PRIESEL, A.: Bindegewebige Gewächse des Eierstockes von besonderer Bauart (Fibroma the cocellulare xanthomatodes ovarii). Beitr. path. Anat. **90**, 199 (1932).
MANSELL, H., HERTIG, A.T.: Granulosa-theca cell tumors and endometrial carcinoma; a study of their relationship and a survey of 80 cases. Obstet. and Gynec. **6**, 385 (1955).
MARSH, J.M., SAVARD, K., BAGGETT, B., WYK, J.J. VAN, TALBERT, L.M.: Estrogen synthesis in a feminizing ovarian granulosa cell tumor. J. clin. Endocr. **22**, 1196 (1962).
MAUVAIS-JARVIS, P., BAULIEU, E.E.: Analysis and means of conjugation of the urinary 17-ketosteroids during virilism. Ann. Endocr. (Paris) **22**, 549 (1961).
MEIXNER, H.: Malignes Arrhenoblastom. Arch. Gynäk. **191**, 31 (1958).
MELLIN, G.W.: Drugs in the first trimester of pregnancy and the fetal life of homo sapiens. Amer. J. Obstet. Gynec. **90**, 1196 (1964).
MEYER, R.: Tubuläre (testikuläre) und solide Formen des Andreioblastoma ovarii und ihre Beziehung zur Vermännlichung. Beitr. path. Anat. **84**, 485 (1930).
— Beitrag zur Frage der Funktion von Tumoren der Ovarien, insbesondere solcher, die zur Entweiblichung und zur Vermännlichung führen. Arrhenoblastome. Zbl. Gynäk. **54**, 2374 (1930).
— Über die Art der zur Vermännlichung führenden Ovarialtumoren. Z. Geburtsh. Gynäk. **98**, 149, 543 (1930).
— Pathology of some special ovarian tumors and their relation to sex characteristics. Amer. J. Obstet. Gynec. **22**, 697 (1931).
MORRIS, J. MCLEAN, SCULLY, R.E.: Endocrine pathology of the ovary. St. Louis: Mosby Comp. 1958.
MOULONGUET, P.: Les diagnostics anatomocliniques de P. Lecène, receuillis par ses élèves. II. Appareil génital de la femme. II. partie. Paris: Masson 1932.
MÜHLBOCK, O.: Neuere experimentelle Untersuchungen über die Genese der Ovarialtumoren. Geburtsh. Frauenheilk. **12**, 289 (1952).
MÜLLER, J.H.: Seltener Fall von verkrebster Struma ovarii in einer Dermoidcyste. Gynaecologia (Basel) **121**, 330 (1946).
— Die Anwendung der künstlichen radioaktiven Isotope in der Gynäkologie und deren Grenzgebieten. In: Klinische Fortschritte — Gynäkologie, T. ANTOINE, Ed. Vienna: Urban & Schwarzenberg 1954.
— Papillares großfollikuläres Schilddrüsenadenom Wegelin in einer Struma ovarii. Schweiz. med. Wschr. **91**, 357 (1961).
NETTER, A.P.: The Stein-Leventhal syndrome. Proc. roy. Soc. Med. **54**, 1006 (1961).
NOKES, J.M., CLAIRBORNE, H.A., RHEINGOLD, W.N.: Thecoma with associated virilization. Amer. J. Obstet. Gynec. **78**, 722 (1959).
NOVAK, E.: Gynecologic and obstetric pathology. Philadelphia: W.B. Saunders 1952.
— In HENDERSON, D.N.: The malignancy of special ovarian tumors. Discussion. Amer. J. Obstet. Gynec. **62**, 824 (1951).
— Masculinizing tumors of the ovary (arrhenoblastoma, adrenal ovarian tumors). Amer. J. Obstet. Gynec. **36**, 840 (1938).
NOVAK, E.R., LONG, J.H.: Arrhenoblastoma of the ovary; a review of the Ovarian Tumor Registry. Amer. J. Obstet. Gynec. **92**, 1082 (1965).
— MATTINGLY, R.F.: Hilus cell tumor of the ovary. Obstet. and Gynec. **15**, 425 (1960).
OVERZIER, C., HOFFMANN, K.: Tumoren mit heterosexueller Aktivität. In: Die Intersexualität, C. OVERZIER, Ed., p. 409. Stuttgart: Thieme 1961.
PEDOWITZ, P., POMERANCE, W.: Adrenal-like tumors of the ovary. Review of the literature and report of two new cases. Obstet. Gynec. **19**, 183 (1962).
PERLOFF, W.H., JACOBSOHN, G.: Effect of HCG on urinary 17-ketosteroids in a patient with Stein-Leventhal syndrome before and after oophorectomy. J. clin. Endocr. **23**, 1177 (1963).
PICK, L.: Über Neubildungen am Genitale bei Zwittern. Arch. Gynäk. **76**, 191 (1905).
PLOTZ, E.J., WIENER, M., STEIN, A.A.: Steroid synthesis in cystadenocarcinoma of the ovaries. Amer. J. Obstet. Gynec. **94**, 189 (1966).
RÜTTNER, J.R.: Zur Morphologie und Histogenese des Arrhenoblastoms unter Berücksichtigung der Hormonanalyse des Tumorgewebes. Schweiz. Z. Path. **20**, 59 (1957).
SAAMELI, K., IKLÉ, F.: Ein Beitrag zur Klinik der Granulosazelltumoren. Gynaecologia (Basel) **148**, 108 (1959).
SAILER, S.: Struma ovarii. A report of 2 cases. Amer. J. clin. Path. **13**, 271 (1943).
SANTESSON, L.: Clinical and pathological survey of ovarian tumours treated at the Radiumhemmet. I. Dysgerminomas. Acta radiol. (Stockh.) **28**, 644 (1947).
SCHICKELE, G.: Adenoma tubulare ovarii (testiculare). Beitr. Geburtsh. Gynäk. **11**, 263 (1907).
SCULLY, R.E.: Androgenic lesions of the ovary. In: The ovary, GRADY, H.G., D.E. SMITH, Eds., p. 143. Baltimore: The Williams & Wilkins Comp. 1963.
SELYE, H.: Ovarian tumors. In: Encyclopedia of endocrinology, Section IV, the ovary; vol. 7. Montreal: Richardson, Bond & Wright 1946.
SIMMER, H., HILLEMANNS, H.G.: Arrhenoblastom und Schwangerschaft. Arch. Gynäk. **196**, 541 (1962).
SJÖSTEDT, S., WAHLEN, T.: Prognosis of granulosa cell tumors. Acta obstet. gynec. scand. **40**, (Suppl. 6), 1 (1961).
SMITH, F.G.: Pathology and physiologie of struma ovarii. Arch. Surg. **53**, 603 (1946).
SOHVAL, A.R., SOFFER, L.: The influence of cortisone and adrenocorticotropin on urinary gonadotropin excretion. J. clin. Endocr. **11**, 677 (1951).
STURLEY, R.F.: Teratomatous chorionepithelioma of the ovary. Critical review of literature with report of a new case. Minnesota Med. **25**, 629 (1942).
TEILUM, G.: Classification of ovarian tumors. Acta obstet. gynec. scand. **30**, 292 (1952).
THOMAS, C.Y., FISHER, E.R., TURNBULL, R.B., KRIEGER, J.S.: Arrhenoblastoma of the ovary. Report of three cases. Ann. Surg. **135**, 543 (1952).
VARANGOT, J.: Les tumeurs de la granulosa. Paris: Librairie Louis Arnette 1937.
WAGNER, G.A.: Die Geschlechtsmerkmale und ihre Beeinflussung durch Tumoren. Zbl. Gynäk. **54**, 1653 (1930).
WERDT, F. v.: Über die Granulosazelltumoren des Ovariums. Beitr. path. Anat. **59** (1914).
WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence. Springfield (Ill.): Charles C. Thomas 1965.

XI. Pregnancy

PAUL J. KELLER

A. Historical Dates

1904 HALBAN indicated the endocrine function of the placenta.
1928 ASCHHEIM and ZONDEK developed the first usable pregnancy reaction.
1930 PHILIPP proved the placental origin of chorionic gonadotropin.
1929–1934 representation and characterization of estrogens and progesterone from urine in pregnancy by BUTENANDT, DOISY, MARRIAN etc.
1958–1970 Exhaustive investigations of steroid biosynthesis in the feto-placental unit by RYAN, DICZFALUSY, ZANDER and many other researchers.
1960 Development of an immunological pregnancy test by WIDE.
1962 JOSIMOVICH isolated and characterized placental lactogen.

B. Anatomy

1. Development and Structure of the Placenta

The ovum migrates into the uterine cavity within 3 to 4 days after fertilization in the tube. During this time, it undergoes continual cell division, becoming converted into a blastocyst, a hollow sphere consisting of the trophoblast and the embryoblast. The placenta develops from the trophoblast, and the embryo from the embryoblast.

After about 6 days, the blastocyst becomes implanted in the converted secretory maternal endometrium by means of proteolytic ferments of the trophoblast. As early as 10 days after conception, the blastocyst is completely surrounded by endometrial tissue, which becomes increasingly loose and vascular under the influence of progesterone, thus developing into decidua.

The trophoblast is then differentiated into two cell layers. The inner layer is termed as the cytotrophoblast or Langhans's layer and consists of cuboid, well-defined cells. The outer layer is called the syncytiotrophoblast, and the borders of its cells cannot be distinguished. Both layers grow rapidly, and 11 to 13 days after conception, primordial villi develop, bounded internally by the cytotrophoblast and externally by the syncytiotrophoblast. The primordial villi open the maternal lacunae in the endometrium, which has by this time been converted into the decidua. The lacunae thus develop into intervillous spaces filled with blood. The extra-embryonic mesoderm supplies the villi with stroma and capillaries which are later connected to the embryonic circulatory system.

The most important organs are formed in the embryoblast between days 15 and 42 after conception. This phase is termed organogenesis. The definitive placenta also develops during this period. The villi adjacent to the uterine cavity degenerate. This area is called the chorion laeve. In contrast, the villi emanating from the decidua basalis of the uterine wall continue to grow rapidly to form the chorion frondosum and hence the placenta.

The mature placenta is oval or circular in shape. It has an average diameter of 15–20 cm. It is 1.5–3 cm thick and weighs between 500 and 600 g with the umbilical cord. It is bounded on its fetal side, where the umbilical cord is attached, by the chorionic plate consisting of trophoblast and the fibrinoid tissue. The uterine surface is bounded by the basal plate, whose structure is similar. The villous tissue is found between these layers and is compressed into the cotyledons and the intervillous spaces perfused by maternal blood (Fig. 1). The villi themselves consist of a fibrous stroma in which the fetal villous capillary network runs. The villi are bounded by syncytiotrophoblast and cytotrophoblast, the latter disappearing almost completely between the 3rd and 5th months (Figs. 2, 3). The total villous surface is estimated to be about 15 m^2 in the mature placenta (WILKIN, 1965).

Oxygenated maternal blood flows through the spiral arteries of the decidua basalis into the intervillous spaces. There, it perfuses the villi and then returns to the circulation by

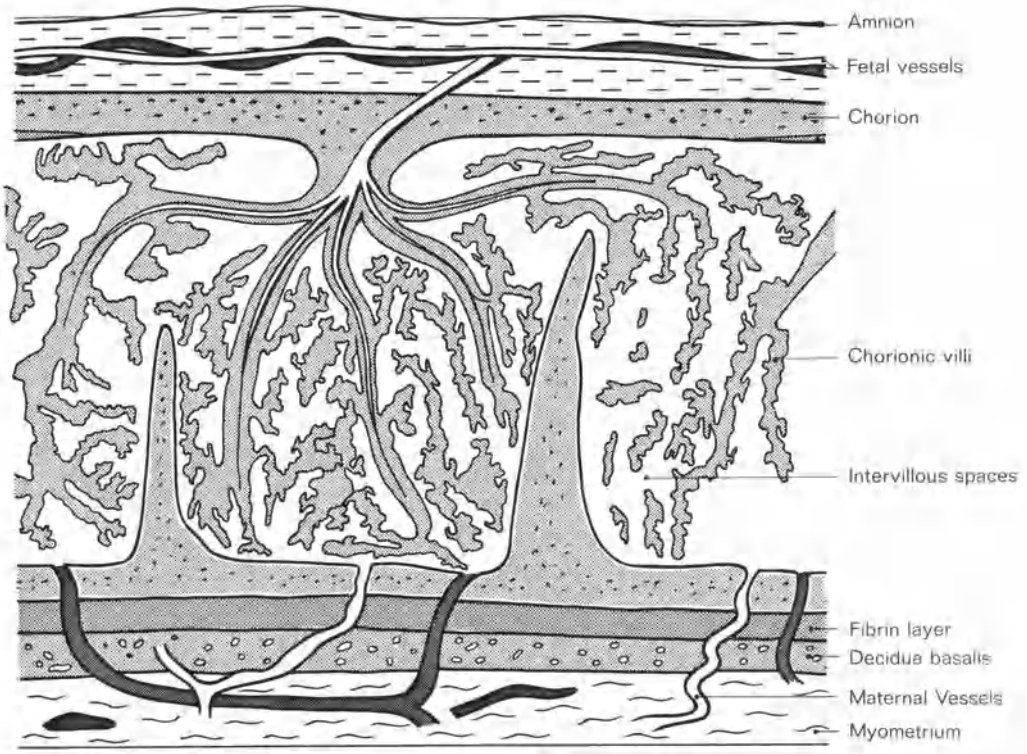


Fig. 1. Schematic representation of the structure of the mature placenta

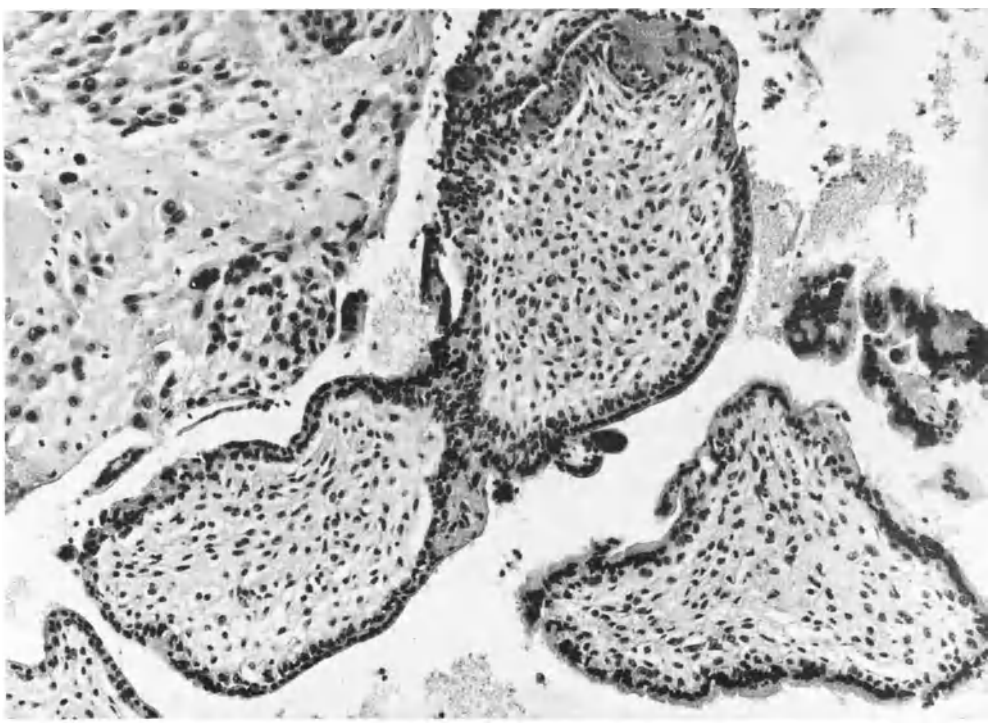


Fig. 2. Histological picture of the chorionic villi during early pregnancy (11th week of pregnancy)

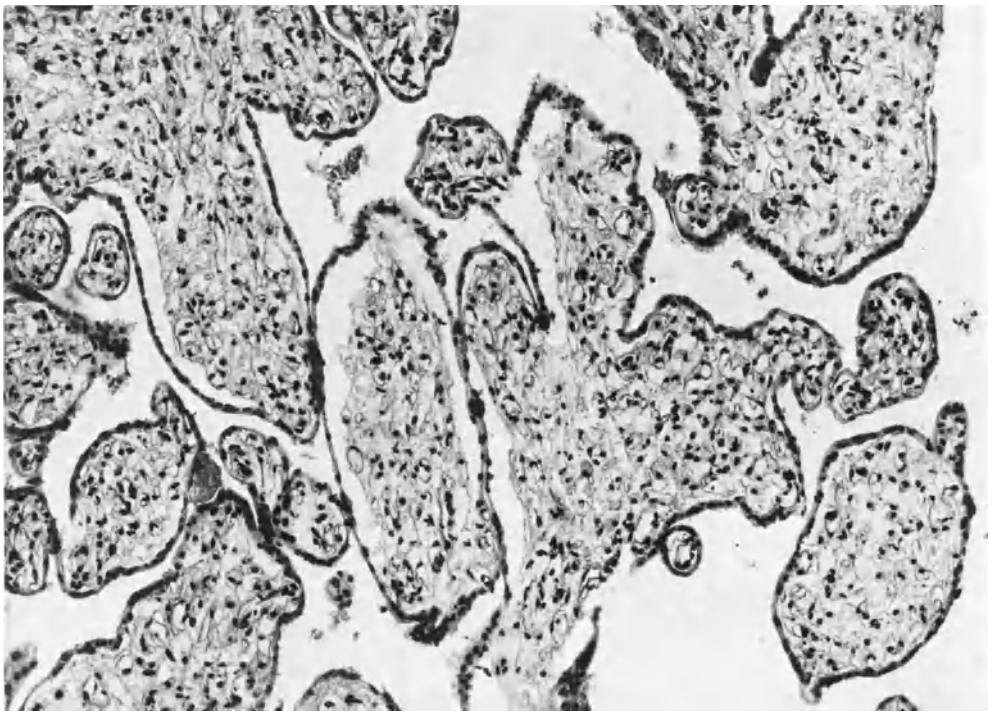


Fig. 3. Histology of the chorionic villi during the last 3rd of pregnancy (39th week of pregnancy)

way of the border sinus and through the veins of the basal plate. Fetal blood flows from two umbilical arteries into the main vessels of the cotyledons and is distributed in the villous capillary system. After being oxygenated, it flows via the umbilical vein back into the fetus. The minute volume of maternal perfusion of the intervillous spaces lies between 375 and 500 ml (BARTELS, 1962; BROWNE, 1954), the minute volume of fetal circulation in the umbilical cord between 150 and 500 ml (GREENFIELD, 1951; ROMNEY, 1955).

Feto-maternal exchange of substances takes place through the syncytiocapillary membrane of the villi. Since the chorion is directly perfused by maternal blood, a hemochorial system is involved. The details of the transplacental transport mechanism have not been explained, but in addition to diffusion processes of varying speeds, active transport and even passage through leaks in the placental membrane may be considered. Apart from this, the trophoblast is also capable of performing a large number of anabolic and catabolic functions, such as the synthesis of fatty acids, proteins, and nucleic acids. Histochemical investigations have facilitated great strides in the relation of individual substances to definite tissue structures.

The fetus is connected to the placenta by the umbilical cord, and is completely mobile in the amniotic fluid surrounded by the fetal membranes. Like the placenta, these membranes consist of fetal and maternal portions, i.e. the amnion, chorion, and decidua.

Details should be obtained from textbooks on obstetrics.

2. Specific Changes of Other Endocrine Glands

a) Hypophysis

The adenohypophysis (anterior pituitary) increases in weight by an average of about 100 mg during pregnancy. The primary histological feature is the large number of pregnancy cells. These cells have large nuclei, stain poorly, and are probably derived from the chromophobe γ cells. The function of these cells has not yet been satisfactorily explained. They are probably an expression of the effect of placental hormones, since similar changes are also found in men suffering from a chorion-epithelioma. These cells regress considerably after the pregnancy.

b) Ovary

The most conspicuous change in the ovary is seen in the corpus luteum, which does not regress after implantation of the fertilized ovum as in the menstrual cycle (see p. 520), but in-

creases in size and is converted into the cystic corpus luteum graviditatis. Histologically, all the signs of an increase in function are present. The layer of theca lutein cells extends and the layer of granulosa cells becomes more vascular. Peripheral vacuolization of the cells increases and more fat globules are deposited in the cells. Degenerative processes begin in the third month of pregnancy; subsequently, the layer of theca cells steadily disappears, the vessels in the layer of granulosa cells recede, and finally, fibrous organization occurs.

Follicular maturation fails to occur throughout the pregnancy due to the absence of rhythmic regulation by pituitary gonadotropins. Nevertheless, a certain degree of follicular growth can sometimes be observed, but the stage of follicular rupture is never reached. Mild cyclic bleeding occasionally arising in the first few months of pregnancy may possibly be due to this state.

c) Adrenal Cortex

The adrenal cortex hypertrophies during pregnancy and there is a measurable increase in weight. Primarily the width of the inner regions of the zona fasciculata (see p. 287) increases. Increased vascularization and the deposition of lipoids can be observed.

C. Biochemistry of Pregnancy- and Lactation-Regulating Hormones

1. Placental Protein Hormones

a) Chemistry

The placenta produces at least two and possibly even several highly molecular protein hormones. Chorionic gonadotropin (HCG, human chorionic gonadotropin) has been investigated most intensively. It is found in large amounts in the placenta, blood and urine even in early pregnancy. The hormone is generally obtained by complicated extraction procedures since it has so far been impossible to synthesize it. The most reliable methods are similar to those used for the extraction of hypophyseal gonadotropins such as alcohol and acetone precipitation, adsorption onto kaolin, permutit or benzoic acid. Additional purification by means of chromatography on ion exchangers such as Decalso, Dowex 2 or Sephadex, and electrophoretic procedures makes a specific activity of 10000–20000 IU/mg possible (Got, 1960; Reisfeld, 1960; Wilde, 1965; Van Hell, 1966). In spite of these astonishing results, there is still no absolute certainty that pure HCG can be obtained. Several compounds fulfil the criteria for homogeneity in electrophoresis, ultracentrifugation and immunochemistry, but even one of the purest fractions seems to consist of different biologically active parts (Van Hell, 1966). In the circumstances, it is not surprising that the chemical constitution has not been fully explained and that there is some controversy about the matter. It has been confirmed that HCG is a glycoprotein and that the fairly high proportion of sugar consists mainly of mannose and galactose (Got, 1960). According to Blobel (1962), there are also 10% hexoses, 12% hexosamine, and 6% neuraminic acid. The last component is of special interest since splitting with neuraminidase or 0.01 N HCl leads to a loss of biological activity. The protein component consists of numerous chains of amino acids, asparaginic and glutaminic acids being the two most predominant. The molecular weight is probably about 30000 (Got, 1959; Nydick, 1964), but figures vary. The isoelectric point lies between 3 and 4 (Got, 1959; Reisfeld, 1960). HCG is very stable in the desiccated form. Heating causes a loss of biological activity. It can also be destroyed by a series of chemicals, particularly by urea.

Placental lactogen (HPL), isolated by Josimovich in 1962, is also of importance. On the basis of its properties, a number of other names have been suggested, such as HCS (human chorionic somatomammotropin) or CGP (chorionic growth hormone prolactin). This hormone can be obtained from homogenized or lyophilized placenta by means of alkaline, aqueous extraction followed by precipitation of the active fraction with ammonium sulfate. Gel filtration on Sephadex (Florini, 1966) is particularly suitable for further purification. HPL is chemically and immunologically closely related to human growth hormone. It is a highly molecular polypeptide. The most important amino acids are again asparaginic and glutaminic acids and leucine. The molecular weight is about 38000 (Florini, 1966; Catt, 1967). Measurements in 5 N guanidine gave rise to values half as high. This could mean that the hormone is present in the dimerous state in aqueous solution. Absolute homogeneity has apparently not yet been achieved.

In addition to these well characterized substances, the placenta also appears to synthesize a thyrotropic protein (HCT, human chorionic thyrotropin) which is chemically quite similar to the corresponding pituitary hormone. Extraction and purification are achieved by methods similar to those used for other placental protein hormones (Hennen, 1969). An exact characterization has yet to be performed.

b) Formation

It is firmly established that HCG arises from the placenta. The most important evidence in support of this fact appears to be provided by the absence of any considerable gonadotropic activity in the hypophysis of a pregnant woman (PHILIPP, 1930), the rapid fall of the HCG titer after removal of the placenta, and the production of this hormone in placental tissue *in vitro* (STEWART, 1948). The question of cellular origin presents more difficulties. Specific histochemical investigations with fluorescent antibodies have led to the conclusion that HCG is formed predominantly in the syncytiotrophoblast although other placental structures also show some degree of activity (MIDGLEY, 1962; LEZNOFF, 1963).

Nor is there any further doubt that HPL is also produced in the placenta. It disappears rapidly after removal of the placenta, and no significant amounts are demonstrable in the hypophysis or fetus. It is produced principally in the syncytiotrophoblast and not in the cytotrophoblast, as has been shown by histochemical studies (SCIARRA, 1963).

Assertions about other proteins hormonal nature which may be present in the placenta seem premature.

c) Metabolism

HCG is primarily released into maternal blood. Minute amounts however, also reach the fetal circulation by routes which are still obscure. It can also be domonstrated in the amniotic fluid and in all maternal tissues. It is excreted mainly in the urine, the average renal clearance lying between 0.38 and 0.95 ml/min (JOHNSON, 1950; LORAINE, 1950). A small amount also appears in the milk. HCG values decrease rapidly after birth (see p. 693) and less than 10% of the antenatal circulating activity can be detected in the urine (WILSON, 1949). The manner of inactivation is unexplained. The liver appears to have no part in this (LEEB, 1956). It seems rather doubtful that postulated inhibitors (SOFFER, 1961) exert any influence.

HPL and HCG are metabolically similar. Most of the activity of HPL is found in maternal serum and in retro-placental blood. Mere traces are the most that can be demonstrated in the fetal circulation (KAPLAN, 1965). The concentration in the amniotic fluid is about 10 to 20 times higher than that in the maternal circulation (KELLER, 1970). The hormone disappears exceptionally quickly after removal of the placenta. The biological half-life is about 20 min (BECK, 1967). Only small amounts of HPL are found in maternal urine, and the renal clearance lies between 0.003–0.007 ml/min (KELLER, 1970). The method of inactivation of this hormone is also unexplained.

2. Steroid Hormones

a) Chemistry

A large number of C_{18}, C_{19} and C_{21} steroids have been demonstrated in the placenta. Among the C_{18} steroids, estrone, estradiol, and particularly, estriol are the most important hormones found. This is a similar state to that in non-pregnant women. Androstenedione and dehydroepiandrosterone are the most important C_{19} steroids, and progesterone and pregnenolone, the most significant C_{21} steroids. The most important structural formulae are presented in Fig. 4. Further details of steroid chemistry and the question of nomenclature, are gone into on p. 288.

b) Formation

Placental steroid hormones are probably produced principally in the syncytial part of the villous system. The complicated process can be investigated by means of incubation experiments *in vitro* which involve incubation of tissue sections or placental homogenates with the suspected precursors in predetermined conditions. The metabolites produced are then analyzed. Perfusion studies are even more conclusive. The placental vascular system is perfused *in vitro* or *in vivo* with the initial material in a suitable medium.

Numerous studies have shown that the placenta produces estrone and estradiol mainly through transformation of C_{19} steroids, particularly dehydroepiandrosterone (DHA), probably formed in the adrenal cortex of mother and fetus. These precursors reach the placenta predominantly in sulfate-conjugated form. There they are hydrolyzed by enzymes, converted into androstenedione and 19-hydroxyandrostenedione by 3β-hydroxysteroid dehydrogenase and 4,5-isomerase, and then aromatized (Scheme 1). The fetus can probably also aromatize very small amounts of androstenedione into estrone and estradiol.

Since the placenta contains only small amounts of 16α-hydroxylase or none at all, the formation of estriol is more complex. This process probably involves 16-hydroxylation of DHA-sulfate in the fetal liver. This substance is then hydrolized again in the placenta and converted into estriol by means of 3β-steroid dehydrogenase, 4,5-isomerase, aromatizing enzymes and 17β-steroid dehydro-

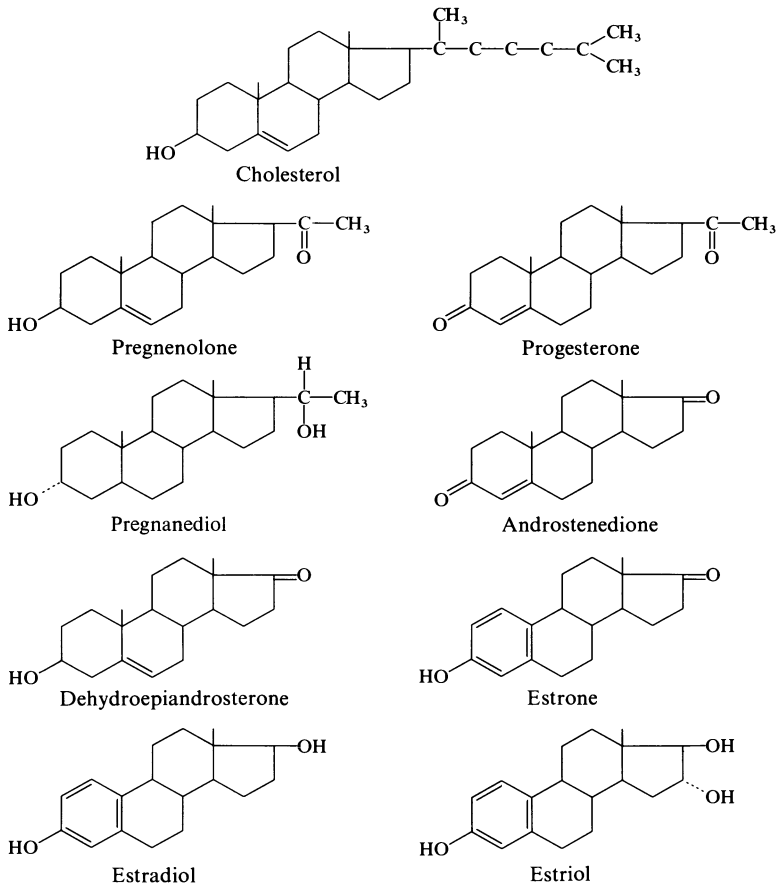


Fig. 4. Structural formulae of some important steroids of the feto-placental unit

genase. Transformation can occur via 16α-OH-androstenedione and 16-OH-estrone or more slowly via androstenetriol and 16α-OH-testosterone (DELL'AQUA, 1967). A second route, also via the fetus, consists in conversion of sulfated estrone present in the placenta by means of 16-hydroxylase into 16-OH-estrone sulfate. This then reaches the placenta again, is hydrolyzed there, and transformed into estriol by 17β-steroid dehydrogenase in the way already described.

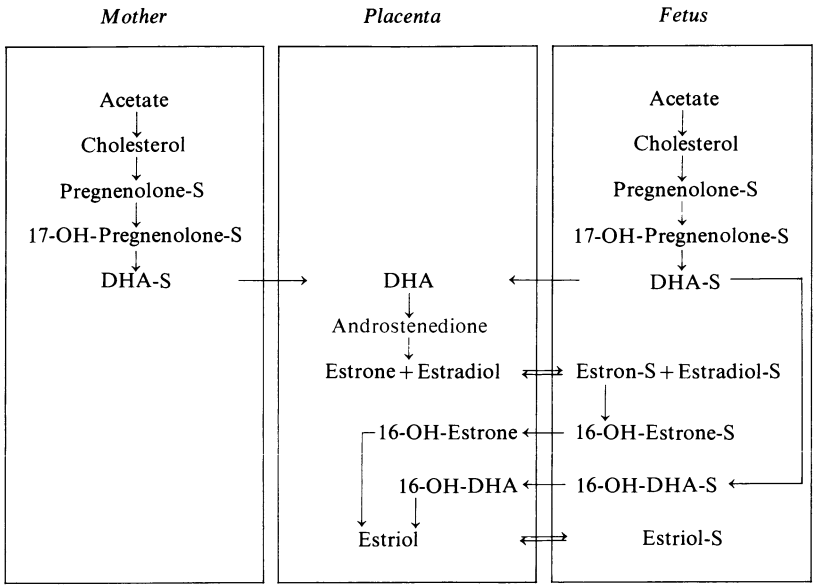
In addition to the placenta, the fetus is probably also capable of producing estrone and estradiol from DHA formed from pregnenolone. Limited amounts of estriol can be synthesized directly.

Progesterone (Scheme 2) is synthesized in the placenta mainly from pregnenolone or pregnenolone sulfate by means of 3β-hydroxysteroid dehydrogenase. Pregnenolone and pregnenolone sulfate are obtained either from the maternal or fetal circulation or are formed from cholesterol in the fetus and mother. Formation of cholesterol from acetate (ZANDER, 1966) is not necessarily of great importance in the placenta. Appropriate hydroxylation processes allow the derivation of small amounts of 20α-hydroxyprogesterone, and probably 6β-16α-, 17α- and 20β-hydroxyprogesterone from progesterone.

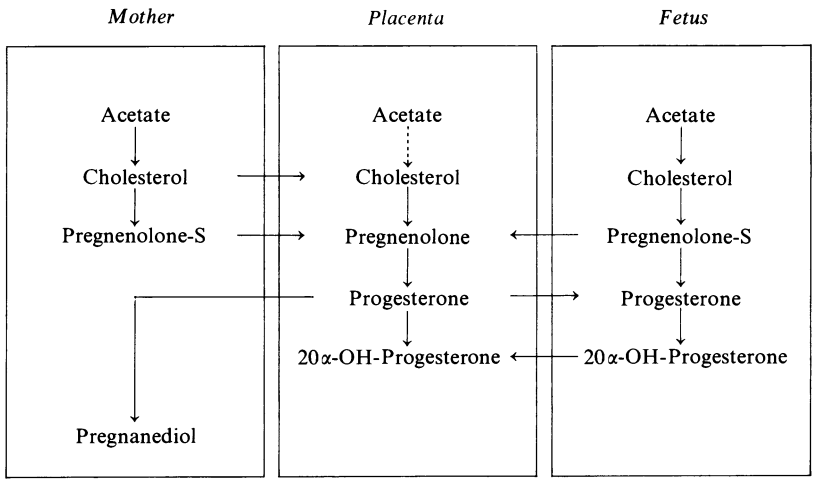
The fetus can produce cholesterol from acetate and is able to convert small amounts in the adrenal cortex into pregnenolone and progesterone. This route is presumably of only slight importance. The maternal production of progesterone is practically insignificant. Neither castration nor adrenalectomy results in a marked fall of progesterone metabolites during pregnancy. (DICZFALUSY, 1961; HAMMERSTEIN, 1965; HARKNESS, 1966).

Androgens found in the placenta, especially androstenedione and dehydroepiandrosterone, are of particular metabolic importance as precursors of estrogens. They are probably produced mainly in the fetal and maternal adrenal cortex.

Scheme 1. Fundamentals of the biosynthesis of estrogens in the placenta



Scheme 2. Fundamentals of the biosynthesis of progesterone in the placenta



c) Metabolism

The metabolism of steroid hormones during pregnancy is also extremely complex since numerous systems are involved at the same time, such as the placenta, adrenal cortex, gonads, fetal liver and maternal endocrinium. Transformation is effected by an extensive enzymatic system including a large number of different hydroxylases, hydroxysteroid dehydrogenases, isomerases, desmolases, sulfatases, sulfurylases, glucuronidases and glucuronylases.

The placenta produces increasing amounts of all the estrogens during the course of pregnancy. Estrone and estradiol can be converted into each other. Both steroids are released into the fetus and sulfated there. They are still partly in the sulfated form when they return to the placenta, where the conjugate is enzymatically hydrolyzed. As has been described already, 16-hydroxylation can also occur in the fetus, permitting subsequent transformation into estriol by the placenta (see p. 671). Only limited amounts of estrone and estradiol are found in the amniotic fluid and meconium. Large amounts of estriol are exchanged between the placenta and the fetal circulation. It also reaches the maternal circulation together with the other two estrogens, but its concentration in the maternal plasma is considerably lower

than that in the umbilical blood. Estriol is present mainly in the form of sulfate in the fetal circulation, whereas it is found mainly as a glucuronide or double conjugate in maternal blood, amniotic fluid, and meconium (TOUCHSTONE, 1963).

Excretion of the estrogens in urine varies. The renal clearance for estrone is 11.9, for estradiol 8.8, and 205 ml/min for estriol (BROWN, 1960, 1964). It is possible that sulfate and free estrogens are not excreted through the renal tubules, or only to a slight extent. This would explain the great differences, since in the plasma, estrone is present predominantly in the free form, estradiol as a sulfo-conjugate, and estriol as a glucuronoside or double conjugate. In addition to the classic estrogens mentioned, at least 20 other estrogenic metabolites can be found in the urine during pregnancy, such as 6α-hydroxyestrone, 15α-hydroxyestrone, -estradiol and -estriol, 15β-hydroxyestrone and -estradiol, 16α-, 16β- and 18-hydroxyestrone, 16-keto estrone and -estradiol, 16-, 17- and 16,17epi-estriol, 2-methyoxyestrone, -estradiol and estriol and others. Fetal urine contains mainly estriol and small amounts of estrone and estradiol (DICZFALUSY, 1957).

Increasing amounts of progesterone are also produced. About a third of it is liberated into the fetal circulation and two thirds into the maternal circulation (ZANDER, 1959). Investigations of the concentrations of progesterone and its metabolites in the umbilical cord have shown that mainly progesterone is present in the vein, whereas 20α-, 20β- and 17α-hydroxyprogesterone are found in the artery. From these findings it can be concluded that the progesterone released from the placenta into the fetus is probably hydroxylized by the fetal adrenal cortex and is returned to the placenta chiefly in the form of 20α-hydroxyprogesterone (ZANDER, 1961; LURIE, 1966). Further, incubation studies have shown that the fetal adrenal cortex can form small amounts of androgens from progesterone over 17α-hydroxyprogesterone and that corticoid synthesis is also possible through hydroxylation processes at C_6, C_{11}, C_{16}, C_{17}, C_{20}, and C_{21}. The placenta, in contrast, is unable to convert progesterone into estrogens or androgens.

Large amounts of the progesterone released into the maternal circulation are deposited in the adipose tissue (HASKINS, 1950; ZANDER, 1959). On the other hand, progesterone is probably converted mainly into pregnanediol in the liver and glucuronized there as well as in the kidneys and peripheral tissues. Excretion occurs in the urine, feces and bile. In addition to pregnanediol, progesterone, 15α-hydroxyprogesterone, pregnenolone, pregnanetriol and other derivatives can be demonstrated in the urine. The conversion rate of progesterone into pregnanediol has been estimated to be 6 to 30% (KLOPPER, 1956; PEARLMAN, 1957).

In the fetus, progesterone is catabolized in the liver mainly into pregnanediol and small amounts of pregnenolone. Pregnanediol, which is probably also sulfated principally in the liver, either returns to the maternal circulation via the placenta or is excreted in the bile and fetal urine und can thus be demonstrated in amniotic fluid (KLOPPER, 1959). In contrast to the situation with estriol, the fetus does not influence progesterone production in the placenta. This has been shown by ligation of the umbilical cord (CASSMER, 1959).

3. Prolactin

Prolactin is thought to be identical to the luteotropic hormone (LTH). Its relation to milk secretion is particularly interesting. The existence of this substance as a separate principle has also been confirmed in the human and in many other mammals. It is possible to extract lactogenic activities from the human adenohypophysis, and to separate prolactin from HGH, which also has lactotropic and luteotropic properties.

a) Chemistry

The most exact information available is that on ovine prolactin, which is extracted from the anterior pituitary lobe by precipitation with alcohol, fractionated salting out, and purification by chromatography on DEAE-cellulose. Chemically it is a polypeptide with an estimated molecular weight of 23300 (DIXON, 1964). Asparaginic and glutaminic acids, and leucine, are the amino acids predominantly present. The isoelectric point is about 5.7. Prolactin isolated from the cow and pig shows similar properties.

b) Formation

Prolactin is formed in the adenohypophysis, possibly in the acidophil cells which can be stained with erythrosine. During pregnancy in mammals, these cells show increasing secretory activity which becomes even more intensified with the onset of lactation. The prolactin content of the anterior lobe of the hypophysis also rises during pregnancy and remains elevated during lactation. The average concentration in sheep is between 30 and 40 IU/mg.

c) Metabolism

Secretion of prolactin, like that of other anterior pituitary hormones, is regulated by the hypothalamus, principally through a prolactin inhibiting factor (PIF, see p. 30). The existence of a corresponding releasing factor (PRF) is still speculative. Low doses of estrogens can stimulate prolactin secretion directly or indirectly via the hypothalamus. Thyroxine also has a similar effect (MEITES, 1966). Progesterone, testosterone, and cortisol are also thought to be able to suppress the prolactin inhibiting factor thus leading to increased prolactin secretion (SAR, 1968).

Demonstration of this hormone (see p. 701) in the blood, and particularly its specificity, presents considerable technical problems. Results so far published must therefore be accepted with reservations, especially results in man. Measurements in sheep, however, have shown that prolactin activity in the serum is slight and is not changed during pregnancy but is greatly raised during lactation in both serum and adenohypophysis. These findings, however, are contradictory (FORSYTH, 1967).

No confirmed evidence is currently available on the manner of excretion and metabolism.

4. Oxytocin

Oxytocin plays a decisive role in the hormonal regulation of birth (see p. 692) and lactation (p. 696). It is an octapeptide with a molecular weight of about 1000. It is probably formed in the ganglia of the supraoptic and paraventricular nuclei from where it passes to the neurohypophysis. The primary effect of oxytocin is to reinforce the contractions of the uterus and of the myoepithelial cells of the mammary glands. Its very rapid breakdown is partly due to an oxytocinase, which is probably produced in the placenta during pregnancy. More details are given on p. 692.

D. Normal Pregnancy

1. Hormone Production in the Placenta

The placenta has numerous functions, gas and energy exchange between maternal and fetal organisms being of primary importance. In addition, the placenta is an extremely versatile endocrine system, which forms numerous steroids and protein hormones from the beginning of pregnancy. Thus, it not only controls pregnancy itself, but is also involved to a considerable extent in the specific changes of the maternal organism. Its efficiency is amazing. Protein hormones now known to be produced autonomously in the placenta exert luteotropic, mammotropic, somatotropic, anti-insulin, and probably thyrotropic effects. Biosynthesis of steroids is also of utmost importance, but in the case of these hormones the fetal-placental interplay is of greater significance.

The most important confirmed or postulated components of the placenta are summarized in Table 1.

Table 1. Survey of placental hormones

Hormones	Formation in placenta	Main actions
Chorionic gonadotropin (HCG)	Confirmed	Luteotropic, influences steroid biosynthesis
Placental lactogen (HPL)	Confirmed	Somatropic, lactotropic, anti-insulin, influences steroid biosynthesis
Chorionic thyrotropin	?	Thyrotropic
Estrogens	Confirmed	Prepares maternal organism for birth and lactation, uterotropic
Gestagens	Confirmed	Prepares maternal organism for birth and lactation, relaxes uterus
Androgens	Confirmed	Intermediate metabolic importance in steroid biosynthesis
Corticosteroids	?	Mineralo- and glucocorticoid
Relaxin (hormone?)	?	Loosening of the symphysis, uterine relaxation

a) HCG

Production, secretion, and excretion of HCG during normal pregnancy have been studied by numerous investigators by means of biological, immunological and radio-immunological methods. The results of these investigations are on the whole consistent. Absolute values, however, are often more difficult to compare. The trophoblast begins to form HCG as soon as 10–14 days after conception, first in small amounts and then in rapidly increasing amounts. After approximately 10 weeks, an average peak value of 450 IU per g placenta is reached (DICZFALUSY, 1953). The titer rapidly falls to less than 50 IU per g in the fourth month and does not alter significantly during the further course of pregnancy (Fig. 5).

HCG levels in the serum behave in a similar manner. The maximum values are again attained between the 8th and 12th weeks and the level then declines rapidly. Relation of the

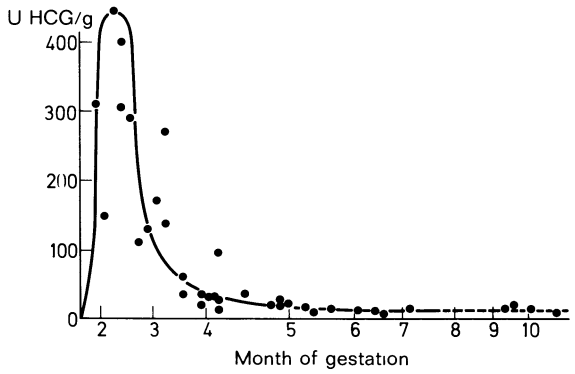


Fig. 5. HCG concentration in the placenta in a normal pregnancy (biologically measured). (After DICZFALUSY, 1953)

level of the titer to the sex of the fetus as postulated by BRODY (1965), appears extremely questionable.

22 to 24 days after conception, or 36 to 38 days after the last menstrual period, the level in the urine is in excess of 1000 IU/l. At this stage, the more sensitive of the conventional pregnancy tests are positive. The amount of HCG excreted in the urine also rises steeply, reaching immunochemical values of 80000 to 240000 IU/l between the 8th and 12th weeks (KELLER, 1966). Between the 13th and 17th weeks, the titer falls just as rapidly and remains between 5000 and 30000 IU/l (Fig. 6) for the rest of the pregnancy. Values can fall below 1000 IU/l in individual cases without any detectable disturbance of fetoplacental function. The striking agreement between placental urinary relations can be deduced from Fig. 5 and 6.

The importance of HCG production with its typical maximum in the first trimester has not been altogether explained. It is assumed that stimulation of the corpus luteum of pregnancy is one of the possible functions of this hormone. Indeed, numerous investigations have shown that HCG has strong luteinizing and luteotropic effects and can prolong the luteal phase in the human. *In-vitro* experiments on the human corpus luteum have also shown that incorporation of C_{14} into progesterone, 17α-hydroxyprogesterone, estradiol and estrone is increased under the influence of HCG (RICE, 1964). A stimulating action on the biosynthesis of progesterone (MASON, 1962) and on the activity of the endopeptidases (JUNG, 1962) has also been demonstrated in animal experiments.

The action on the placenta itself has only been explained to a small extent. Nevertheless, perfusion studies have shown that HCG stimulates the transformation of 16-hydroxylized C_{19} steroids into estriol (TROEN, 1961; 1962; VARANGOT, 1962). The influence of HCG on steroid biosynthesis can be considered as proven. It is still not possible to say with certainty whether the fetal endocrine system is also subjected to a stimulating influence, and if so, to what extent. It appears, however, that the adrenal cortex, testes, and ovaries can be influenced. HCG production is largely independent of fetal function. Ligature of the umbilical cord in utero does not diminish the titer (CASSMER, 1959).

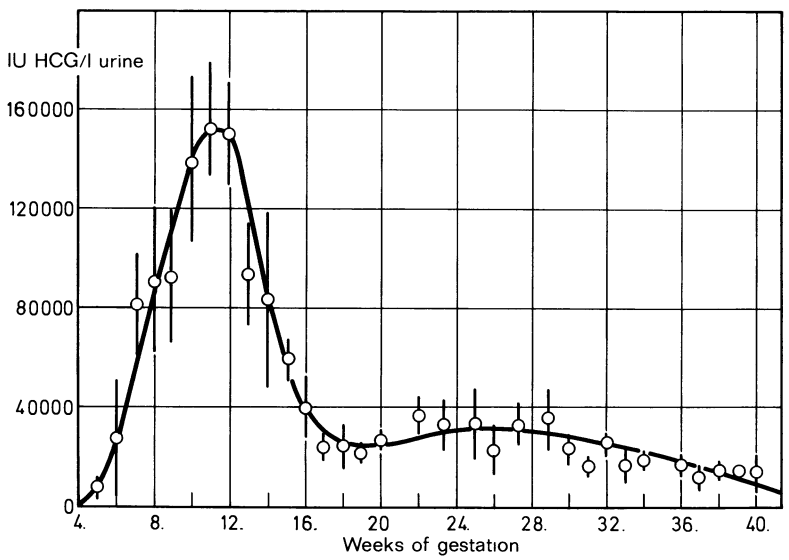


Fig. 6. Urinary HCG excretion during normal pregnancy, average values and standard deviations. (Immuno-chemical assay, after KELLER, 1966)

The question of regulation of HCG production is just as difficult as the question about the meaning of the production. The existence of a feedback mechanism similar to that between sex hormones and the release of hypophyseal gonadotropins is somewhat questionable although various authors have thought that they observed a discrete fall in the HCG titer under the influence of synthetic estrogens and gestagens. Positive and negative effects of estriol and dehydroepiandrosterone have been postulated (LAURITZEN, 1966). Whether large amounts of HCG inhibit continued production also appears questionable. Nevertheless, a slight fall has been reported after injection of HCG (DICZFALUSY, 1956). Precise methods of estimation may solve some of these problems in the next few years.

b) HPL

Human placental lactogen is demonstrable in the trophoblast as little as $3\frac{1}{2}$ weeks after fertilization and its concentration remains more or less constant (1–10 g per 100 g placenta) throughout pregnancy (JOSIMOVICH, 1964). In contrast, plasma levels continue to rise during the course of pregnancy (Fig. 7). Plasma HPL can generally first be detected between the 7th and the 8th weeks. Average values reach a level of 0.5 µg/ml in the 12th week, 2.5 µg/ml in the 24th week, and 6 µg/ml after the 35th week (KELLER, 1970). Only very little HPL is excreted in the urine. The daily production at the end of pregnancy is estimated to be 0.3–1 g.

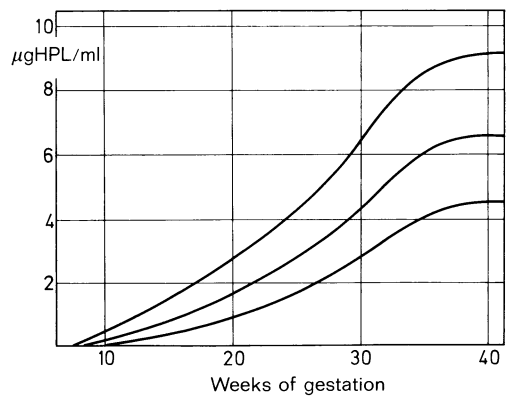


Fig. 7. HPL concentration in serum during normal pregnancy, average values and normal 95% range. (Radioimmunoassay after KELLER, 1970)

The function of placental lactogen is also difficult to define. The fact that only traces of this hormone can be demonstrated in the fetal circulation leads to the conclusion that the main effect must be exerted in the placenta and maternal organism.

There is no doubt that HPL has a weak somatotropic action. This can be shown by animal experimentation, both in the tibia test and in the uptake of labeled sulfate in the costal cartilages of hypophysectomized rats (KAPLAN, 1964). It also induces a general increase in the weight of mice and rats (FRIESEN, 1965). In experimental conditions, nitrogen retention in the human appears to be less pronounced under the influence of HPL than under that of growth hormone. In contrast, high doses of HPL cause a rise in free fatty acids (GRUMBACH, 1966). In addition to its action on fat metabolism, HPL also has a definite diabetogenic, anti-insulin effect on sugar metabolism. This may explain the deterioration of latent diabetes during pregnancy (see p. 801). The physiological significance of this function may be associated with an increase of glucose supply in the pregnant organism through mobilization of free fatty acids (GRUMBACH, 1966).

Like human growth hormone, HPL also exerts a prolactin-like, mammotropic effect, as can be demonstrated on the crop sac of the pigeon and in the secretion of milk in pregnant and pseudo pregnant rabbits. The lactogenic action can also be seen in tissue cultures of the milk glands of mouse, where synthesis of casein is promoted in the presence of insulin and cortisol and the number of cells increases at the same time (TURKINGTON, 1966). The action of HPL is very similar to that of prolactin in animal experiments.

The luteotropic action is another property of this hormone. In animal experiments it can be potentiated by HCG (JOSIMOVICK, 1966). Nothing can be said about this action in the human at present.

The influence on the placenta and its production of steroids is still controversial, but can be considered likely. Stimulation of the conversion of 16-OH-dehydroepiandrosterone into estriol has been demonstrated (TOMINAGA, 1967).

c) Other Protein Hormones

Most recent investigations have shown that, in addition to the two well-characterized placental protein hormones already mentioned, there may be a chorionic thyrotropin (HCT, human chorionic thyrotropin) which may be responsible for the relatively high thyrotropic activity of the serum in pregnancy (HENNEN, 1969).

Placental extracts also contain adrenocorticotropic activity (JAILER, 1950; NEHER, 1961), but

this probably arises from the maternal adenohypophysis rather than from the placenta itself.

Relaxin is another substance which can be extracted not only from the placenta, but also from the uterus and ovaries (HISAW, 1950; ZARROW, 1958). It is a polypeptide which is claimed to cause a loosening of the pelvic ring as well as relaxation of the uterine musculature and dilatation of the cervix. The hormonal nature of this substance is doubtful.

d) Estrogens

During pregnancy the concentration of estrogens rapidly increases in the placenta, maternal blood, and urine. The three classic estrogens are present predominantly in the free form in the placenta. Data about the amounts vary. According to DICZFALUSY (1961), at the end of pregnancy there are 37–64 µg estrone, 3–142 µg estradiol and 125–287 µg estriol per kg of fresh weight. A considerably smaller amount is present in the conjugated form. In addition to the estrogens mentioned, 16-epiestriol, 17-keto estradiol and 6-methoxyestrone have been isolated from the placenta. The rate of secretion of total estrogens has been estimated to be 1 mg in the 10th week, 20 mg in the 20th week and between 40–100 mg at term (BROWN, 1957).

More is known about the behavior of estrogens in the blood. After conception there is no initial premenstrual fall. Levels then rise continuously, until at term the concentration of estrone, estradiol and estriol is found to lie between 2 and 26, 1 and 5, or 3 and 30 µg/100 ml plasma, depending on what technique is used. The quotient

$$\frac{\text{estriol}}{\text{estrone} + \text{estradiol}}$$

rises during the course of pregnancy and is about 4 at term. Similar values of estrone and estradiol are found in fetal blood, but there is a preponderance of sulfo-conjugated estriol so that the above quotient is about 30.

The excretory relations are of special importance for diagnostic reasons to be discussed later. Again, there is no premenstrual fall after conception, and a mid-cyclic titer is attained 14 days after fertilization (Fig. 8). The ratio

$$\frac{\text{estriol}}{\text{estrone} + \text{estradiol}}$$

rises from approximately 1 to 10 from the beginning of pregnancy to term, with a 1000-fold increase in the excretion of estriol during this period and a mere 100-fold increase in the excretion of estrone and estradiol (Fig. 9).

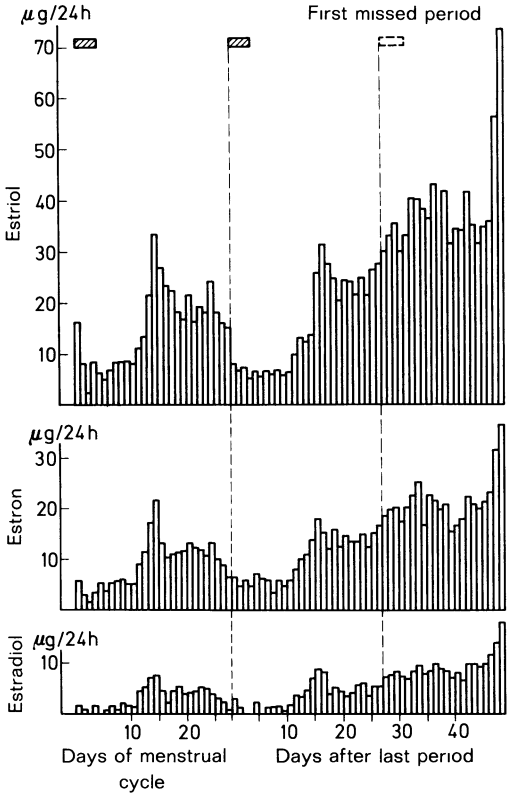


Fig. 8. Urinary excretion of estrone, estradiol and estriol before and after conception. (After BROWN, 1956)

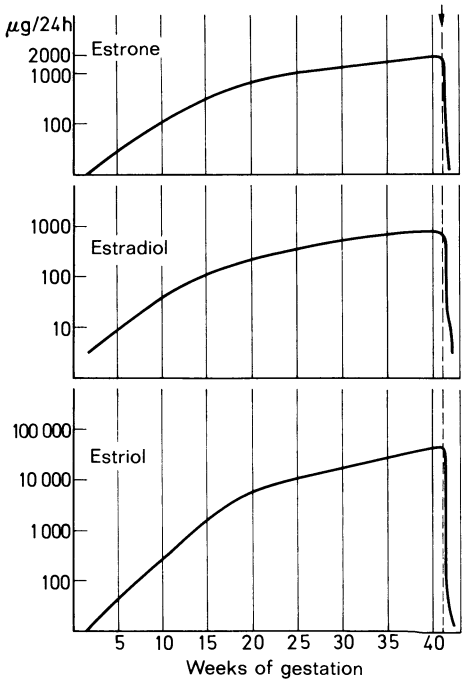


Fig. 9. Excretion of estrone, estradiol and estriol during a normal pregnancy. (After original values from BROWN, 1956)

This is probably due to renal factors as mentioned above.

The physiologic importance of the enormous amounts of estrogens during pregnancy has not been fully explained. A primary factor may be the growth-promoting action on the uterus by increasing protein synthesis. Furthermore, it is known that electrolyte shifts at the cell membrane of the myometrial cell occur together with changes in membrane potential. These are important for contractility. Estrogens also have a considerable effect in the preparation of the organism for lactation (see p. 694).

e) Progesterone

The concentration of progesterone in the placenta also increases during pregnancy, reaching an average value of 1.9 mg/kg at term. This value is lower than concentrations found in earlier stages of pregnancy, but the total progesterone increases (Fig. 10). In contrast, the myometrial concentration rises throughout pregnancy. The secretion rate is estimated to be between 30–250 mg/24 h in the first two trimesters and between 190–840 mg/24 h in the last trimester (SALOMON, 1962; TAUBERT, 1963; RYAN, 1966; ZANDER, 1967). A large amount of the progesterone reaching the circulation is stored in the adipose tissue.

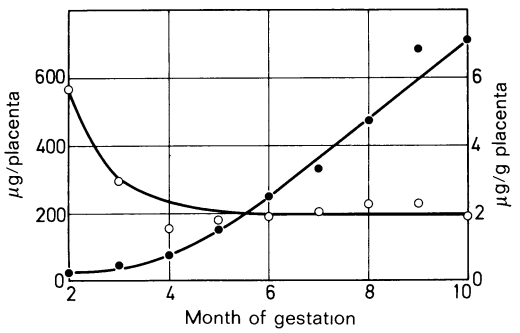


Fig. 10. Total amount of progesterone and progesterone concentration per g placenta during normal pregnancy; average of 80 estimations. (After ZANDER, 1956)

The level of progesterone in maternal blood also rises considerably. Values lie between 5 and 7 µg/100 ml plasma in the first trimester, between 2 and 10 µg/100 ml in the 2nd trimester, and between 4 and 36 µg/100 ml plasma in the last trimester (SHORT, 1959; 1962; VAN DER MOLEN, 1963; AITKEN, 1958). The highest concentrations are found in retro-placental blood and in the umbilical vein. In the umbilical artery, there is a predominance of progesterone metabolites, hydroxylized at positions 20 and 17. These metabolites have a less pronounced progestational action (see p. 674).

As in the non-pregnant state, pregnanediol, principally in the glucuronidized form, is the most important excretory product of progesterone (see p. 674). Excretory values rise from 4–8 mg/24 h at the beginning of pregnancy to 20–80 mg/24 h at term (Fig. 11).

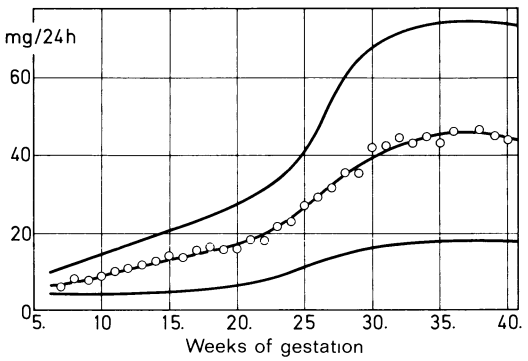


Fig. 11. Pregnanediol excretion in urine during normal pregnancy, average and normal 95% range. (After SHEARMAN, 1959)

The physiological significance of progesterone and its metabolites in the human also remains obscure. On the basis of animal experiments, it is assumed that their primary function is to preserve pregnancy by maintaining uterine relaxation despite neurohypophyseal influences (see p. 692). Furthermore, together with estrogens, they play an important part in preparing the maternal organism for birth and lactation (see p. 694).

f) Androgens and Corticosteroids

As was seen in the description of the formation and metabolism of placental steroids, androgens are important as intermediate products in the biosynthesis of estrogens. This is especially applicable to dehydroepinandrosterone and androstenedione. Adrenosterone and 11β-hydroxyandrostenedione have also been demonstrated in umbilical blood (ZANDER, 1962). Cortisol can be found in the placenta and in umbilical blood (NEHER, 1961). There is no doubt that the fetal adrenal cortex is capable of forming cortisol from progesterone, and possibly from acetate, even in the first half of pregnancy. The cortisol so formed reaches the placenta and the maternal circulation. On the other hand no definite evidence of direct biosynthesis in the placenta has yet been produced (DICZFALUSY, 1964). This is also true of other corticosteroids, although it is possible to isolate small amounts of aldosterone from the placenta (BERLINER, 1956).

2. Feto-Placento-Maternal Hormone Exchange

The maternal and fetal compartments and the placenta form an endocrine unit which can only be understood as a whole, especially in relation to the steroid hormones (Scheme 3).

Maternal protein hormones as a rule do not pass through the placental membrane into the fetus, or only in minute amounts. Potent amounts of TSH and ACTH are hardly likely to reach the fetal circulation (TOROK, 1951; KNOBIL, 1959). The same applies to HGH (GITLIN, 1965; LARON, 1966). HCG can be demonstrated but its concentration in the fetus is significantly lower than that found in maternal blood and tissues. HPL is found virtually only in the placenta and the maternal organism. Insulin does seem to pass the placental barrier to some extent, but concentrations in umbilical blood are substantially lower than those in the mother (JOSIMOVICH, 1961).

Hormones from the thyroid gland and corticosteroids (MIGEON, 1956) reach the fetus only very slowly (GRUMBACH, 1956). The clinical improvement observed during pregnancy in patients with Addison's disease has led to the conclusion that these steroids may also be liberated from the fetus and pass to the mother. There is no doubt that large amounts of androgens can be transferred to the fetal circulation, where they can result in virilization of the female fetus.

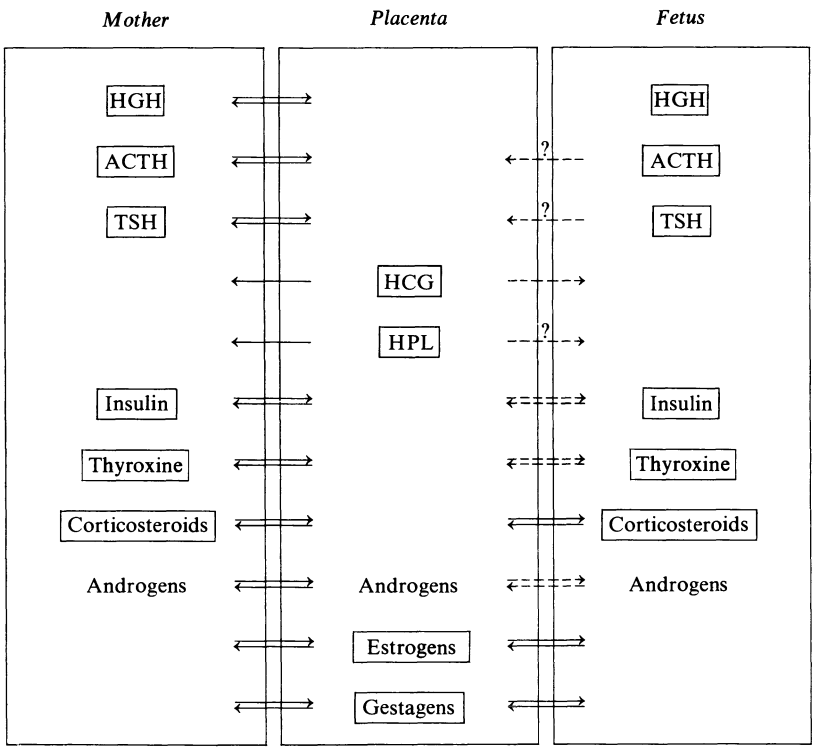
Relationships between the remaining sexual steroids are considerably more complicated. These hormones have been described in section C. Estrogens and progesterone circulate freely between the fetal, placental, and maternal compartments. Since the placenta is capable of producing progesterone independently but cannot form any significant amounts of estrogens, particularly estriol itself, this exchange has a very important effect on the biosynthesis of steroids. The fetal compartment contains precursors of estrogens, such as dehydroepiandrosterone and is also capable of 16-hydroxylation. Steroids are generally present in the conjugated form in the maternal and fetal systems and in the free form in the placenta.

3. Function of Other Endocrine Glands during Pregnancy

a) Hypophysis

Placental steroids inhibit gonadotropin production of the adenohypophysis during pregnancy (PHILIPP, 1930; BETTENDORF, 1966). Recently, however, there has been radio-immunological evidence of FSH-activity in the serum so that some reservation is still indicated before

Scheme 3. Simplified representation of the feto-placento-maternal hormone exchange



a definite conclusion is possible. Formation of TSH, ACTH, HGH, and MSH as a whole may be increased, as suggested by the intensive activity of the thyroid gland, enlargement of the adrenal cortex, and the acromegalic features occasionally observed. The exact extent of the increase in prolactin formation (see p. 674) during pregnancy is not known. Similarly, no definite facts can be given about the function of the neurohypophysis and the secretion of vasopressin and oxytocin during pregnancy.

b) Ovary

The corpus luteum of pregnancy is capable of producing both estrogens and progesterone from cholesterol. The average progesterone concentration is about 12 μg/g. Lutein cells may also produce active androgenic hormones and, in rare cases, hyperplasia of these cells gives rise to transitory virilization.

The hormonal function of the corpus luteum may be involved in the preservation of pregnancy in the early months. In the human, the trophoblast is capable of synthesizing adequate amounts of progesterone at a very early stage. In certain types of animals, such as the cow, rabbit, and rat, in contrast, the corpus luteum is essential for the preservation of pregnancy.

c) Adrenal Cortex

The levels of 17-hydroxycorticosteroids in the plasma rise during pregnancy (GEMZELL, 1953). It is not certain how far this rise can be attributed to increased function of the maternal adrenal cortex. As has been mentioned above, the clinical improvement in patients with Addison's disease during pregnancy suggests that the fetus, or less probably the placenta, is actually involved in the production of corticosteroids.

The level of cortisol also rises, but this is probably mainly because estrogen induces increased binding to a plasma globulin called transcortin (SANDBERG, 1959). This results in extension of the biological half-life, with a resultant rise in the total amount of cortisol. Since cortisol bound to transcortin appears to be biologically inactive, no clinical signs of hypercorticism normally appear during pregnancy. No cortisol can be demonstrated in women with adrenocortical insufficiency, and extra-adrenal sources therefore appear to have no measurable effect (BAULIEU, 1957).

Production and secretion of aldosterone are also increased by as much as ten times during pregnancy (VAN DE WIELE, 1960; STARK, 1963). This rise fails to occur in women who have undergone adrenalectomy (LAIDLAW, 1958), so that it can be assumed that the maternal adrenal cortex is the chief producer of aldosterone in these cases as well.

An adrenal cortex with absolutely intact function is not essential for conception and the preservation of pregnancy. Pregnancy can continue to term without complications even in women with clinically manifest hypofunction.

d) Thyroid Gland

The thyroid gland increases considerably in size during the course of pregnancy and more thyroxine is formed (FREEDBERG, 1957). The concentration of thyroxine in the plasma also rises, but as in the case of cortisol, this rise is primarily due to an estrogen-induced increase in the binding of thyroxine to plasma proteins.

Severe hypo- and hyperfunction of the thyroid gland can have only negligible effects on pregnancy since sterility is present in most of these cases.

4. Hormonal Effects on the Maternal Organism

The enormous production of sexual steroids and protein hormones by the placenta leads to a series of changes in the genital and extragenital regions, which are directed partly at metabolic adjustment to pregnancy and partly at preparation of the organism for birth.

a) Uterus

The weight of the uterus increases 12 to 20 times and its internal volume increases 600 to 800 times (CRETIUS, 1967). The increase in size is due mainly to hypertrophy of muscle cells already present, and less to hyperplasia, which is only of importance during the early stages of pregnancy. Muscle cells may undergo a tenfold increase in length. Animal experiments have shown that the effects of estradiol are virtually limited to hypertrophy, estradiol and progesterone together have a hyperplastic action, and progesterone alone has practically no effect (BRODY, 1961). Thus, hormonal regulation of uterine growth appears to be complex. There is an accumulation of contractile proteins in the myometrium and the energy donors, such as ATP, creatine phosphate and glycogen, increase under the influence of estrogens. The ionic content is subject to displacement, which is partly responsible for the motility of the uterus and is most probably also due to the action of steroids (see p. 692). The myometrial

water content increases, resulting in a rise in tissue fluid.

The vascular system of the uterus also shows characteristic changes. The spiral arteries become stretched and widened and there is a true increase. The vessel walls become hypertrophic. Vascularization rises from 50 ml/min to 500–750 ml/min (CRETIUS, 1967).

The mucosa of the body of the uterus is converted from the secretory stage into the true decidua, mainly under the influence of progesterone and the synergistic action of estrogens. The decidua thickens very quickly and is differentiated into the superficial stratum compactum and the stratum spongiosum lying beneath. Thus, there is extensive enlargement of the glands, which also show signs of increased secretion, and the interstitial tissue is considerably displaced.

The same hormonal influences produce changes in the cervix. The connective tissue becomes loosened, and vessels increase in number and size. Hyperemia of the venous network presents clinically as lividity. Cervical glands proliferate internally, branch, and penetrate into the connective tissue. They secrete thin clear mucus which does not usually show the arborization phenomenon on drying.

b) Vagina

As in the cervix, the tissues in the vagina become looser, which helps to ease the process of birth later on. Width, length, and distensibility increase, vascularization becomes more marked and the veins become dilated. The thickness of the vaginal epithelium increases, principally under the influence of estrogens. The intermediate layer in particular becomes thicker. Desquamation of cells is accelerated and vaginal secretion is increased. Cells in the vaginal smear are predominantly from the intermediate layer.

Vulva, perineum, and pelvic floor show similar signs of loosening.

c) Mammary Glands

Hormonal preparation of the mammary glands for lactation is dealt with in Section G.

d) Skeleton

All joints in the pelvic region become softened during the course of pregnancy under the influence of the sexual steroids. Changes are most marked in the symphysis, which becomes several millimeters wider. These processes can also be considered to be directed mainly at easing the birth. It is not clear at present to what extent other factors, for example relaxin (see p. 678) are involved in this process.

e) Skin

The skin also shows a series of changes in pregnancy, some of which are due to the action of hormones. Accentuated pigmentation is seen in 75% of women towards the end of pregnancy, especially in the regions of the breasts, linea alba, vulva and perineum. This may be quite pronounced in brunettes. Yellow or brown patches often form on the face. This is termed chloasma. These changes are due to the action of estrogens and possibly also to the increased release of melanophoric-stimulating hormone (MSH) from the adenohypophysis. According to ROTHMAN (1954), progesterone is able to activate this hormone and possibly also the melanocytes directly.

Striae gravidarum are observed in many pregnant women, particularly on the abdomen, but also on the breasts and buttocks, and can be attributed to mechanical over-distension as well as to constitutional predisposition and the action of corticosteroids.

Vascular changes are quite common and can take the form of teleangiectatic, spidery vascular patterns on the legs, upper arms and occasionally the face. These may be due to the action of estrogens.

f) Gastrointestinal Tract

Peristalsis is often reduced in the early months of pregnancy. There is a tendency to constipation. Progesterone may be of importance as well as parasympathetic adaptation, since it generally reduces the tone of smooth muscle. Similar features have also been observed in the gallbladder, the ureters, and the renal pelvis.

g) Water Balance

Estrogens cause retention of sodium and water and increase vascular permeability. As in the premenstrual phase, they act with progesterone to produce loosening of the tissues, and the extracellular fluid increases by 2–4 liters. About two thirds of this amount are found in the interstitial tissue space where the water-binding capacity increases. Thus, even in normal pregnancy, there is an increased tendency to edema.

h) Basal Temperature

The basal temperature (see p. 567) remains in the hyperthermal region for 3–4 months after the onset of pregnancy. This is due to the action

of progesterone. The temperature later falls despite the steady increase in the production of progesterone. The reasons for this are unknown, but the temperature remains low until birth occurs. Absence of menstruation in the presence of a raised temperature on waking generally suggests early pregnancy.

E. Pathology of Pregnancy

1. Abortion

a) Terminology

Abortion or miscarriage signifies the premature termination of pregnancy during the first 22 weeks. The fetus is usually not viable. A distinction is made etiologically between spontaneous, legal therapeutic, and criminal abortion. Mild bleeding with an undilated cervical canal is termed threatened abortion. When the cervical canal begins to dilate, the abortion is incipient and can usually no longer be prevented. The products of conception are subsequently either partially or completely expelled, i.e. the abortion is incomplete or complete. During the early weeks of pregnancy, this process occurs in one stage. Later, it occurs in two stages, as at birth, with expulsion of the fetus first, followed by the placenta. Occasionally, the rejected fetus is retained and the abortion is then termed a missed abortion. If three or more abortions occur in succession, the term habitual abortion is used. An abortion can be complicated by fever and bacterial invasion of the tissues, especially after illegal intervention, resulting in a febrile or septic abortion.

b) Frequency

Abortion, which is most frequent during the 2nd and 3rd months of pregnancy, is one of the most important complications of pregnancy. Exact statistical data are difficult to obtain since the number of clinically undetected cases remains unknown. It is generally estimated that about 10% of the total number of pregnancies end in spontaneous abortion. Illegal abortions are, however, much more significant since they are estimated to be up to four times more frequent than spontaneous abortions on average, depending on the country. The frequency of habitual abortions is very low and its incidence is estimated as one in every 300 to 400 births (KÄSER, 1961).

c) Pathogenesis

Pathogenic factors causing abortion can be divided into maternal and fetal factors. In the former group, intrauterine changes are of primary importance. Thus, malformations such as bicornuate uterus, abnormal positions, fixed retroflexion, fibroids, cervical incompetence or genital hypoplasia can be responsible for an abortion. Infections in the mother, severe generalized ilnesses, accidents, toxic states, and even psychological factors are also of significance.

Among the fetal causes, abortive ova are particularly important. Malformations of the embryoblast and trophoblast are often combined. According to HERTIG (1949, 1956), malformations can be demonstrated in 50% of young ova not implanted. Even after implantation, malformations are found in 35%. Chromosomal aberrations are of considerable importance as an etiological factor and can be found in 20–25% of early abortions (KERR, 1966). Trisomics, X-monosomics and triploids are particularly common. Sperm anomalies are probably also important. According to JOEL (1962), highly pathologic spermiograms are found in 5.8% of cases of habitual abortion.

The part played by endocrine factors is still difficult to assess. Most of the changes demonstrated in the hormonal pattern could be of secondary nature and could be an expression of damage to the trophoblast or embryoblast or both. Falls in the levels of HCG, HPL, estrogens and progesterone also permit of this interpretation. Nevertheless, it is still assumed that a hypofunctional corpus luteum graviditatis during early pregnancy, insufficient preparation of the uterus through sexual steroids, and thyroid activity may be of significance.

d) Methods of Diagnosis

Abortion is diagnosed primarily by clinical means. However, hormonal diagnostic methods can be of additional use since in doubtful cases information on the state of the trophoblast may be obtained.

Estimation of chorionic gonadotropin in the plasma and urine is most important. Almost normal titers are characteristic of an imminent abortion as long as pregnancy is still intact. The prognosis is favorable if the HCG value is normal. If considerable damage has already occurred in the trophoblast, the HCG titer is extremely low (Fig. 12). Values below 1000–2000 IU HCG/l urine at the time of the physiological maximal excretion, between the 7th and 12th weeks of pregnancy, suggest that conservative treatment is useless. The titer is also quite low in comparison to the duration of amenorrhea in missed abortion. Surviving placental tissue can, however, still result in positive pregnancy tests for some time.

The excretion of estrogens and pregnanediol is also reduced in cases of abortion. The practical significance of such measurements is limited in contrast to results obtained in the 2nd and 3rd trimesters.

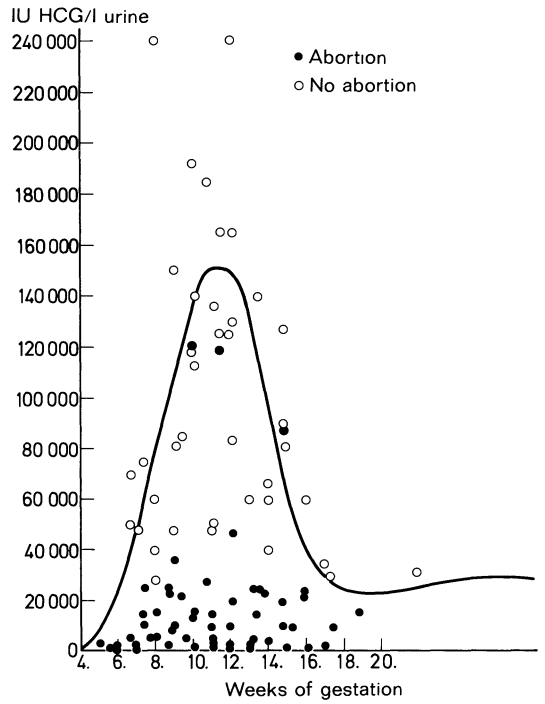


Fig. 12. HCG excretion in urine in cases of imminent abortion with favorable and poor prognosis (immunochemical assay). The curve corresponds to average normal excretion. (After KELLER, 1966)

e) Treatment

Treatment of an abortion with hormones is, of course, only indicated if the pregnancy is still intact, i.e. in a case of threatened abortion. Hormones can also be used prophylactically in habitual abortions and occasionally where pregnancy is exposed to extreme risks or during surgery. Gestagens in high doses are used in these cases, sometimes in combination with estrogens, in the hope that they will have a favorable effect on the endometrium and cause the myometrium to relax (see p. 691). The effect of such measures is, however, still doubtful. Numerous drugs can be used, but care must be taken to ensure that steroids with intrauterine virilizing effects are avoided. Allylestrenol (Gestanon) can be particularly recommended for oral use. It is a very potent gestagen which is thought to have a placentotropic action. A dose of 15–30 mg daily, divided into three doses should be taken for about a week in a case of threatened abortion. In habitual abortion, 5–10 mg must be given daily as a long-term treatment as soon as possible after menstruation is missed. This treatment should be continued for several weeks over the critical point, i.e. to the time at which previous pregnancies were lost. Progesterone can also be given intramuscularly, for example as Lutocyclin in oily solution or crystalline suspension. The daily dose should be between 25 and 100 mg in a case of threatened abortion. If the crystalline suspension is used, weekly injections can be given. Intramuscular injections of 250 mg 17α-hydroxyprogesterone capronate (Proluton-depot) every 2nd or 3rd day have proven very satisfactory. This compound can also be used in combination with an estrogen, for example estradiol valerianate in the form of Gravibinon. 1 ml of this drug (250 mg 17α-hydroxyprogesterone capronate, 5 mg estradiol valerianate) should be given daily over a week in threatened abortion. 2 ml weekly are sufficient in later stages and in cases of habitual abortion, where treatment must start as soon as possible and be continued long after the period which is critical in each case individually.

General management and strict bed rest are even more important than all therapeutic hormonal measures. Spasmolytics such as benzodiazipines (e.g. Valium) can be used as a supportive measure. Surgical procedures such as cerclage can be considered for cervical incompetence. Success of this treatment is often difficult to assess and depends on the pathologic factors already described.

2. Extra-Uterine Pregnancy

In an extra-uterine pregnancy the fertilized ovum becomes implanted outside the uterine cavity. The most common sites are the ampullary and isthmus of the tube. Formation of folds after specific or nonspecific inflammatory processes, a diverticulum, endometriosis and possibly also impaired motility of the tubes are pathogenic factors coming into consideration. The incidence of extra-uterine pregnancy is calculated as one in every 100 pregnancies.

Due to lack of space, developmental disorders usually arise very early with rupture of the fetal membranes. If the membranes rupture inwards tubular abortion occurs, while outward rupture of the membrane results in tubular rupture, which is much more dangerous and potentially fatal.

The diagnosis is made clinically, but hormonal investigations can occasionally be of use. Since the placenta is relatively small and there is also placental damage at the time clinical symptoms arise, the HCG level in the

serum and urine is usually considerably reduced (Fig. 13). Conventional pregnancy tests are therefore often negative.

Surgery is usually necessary.

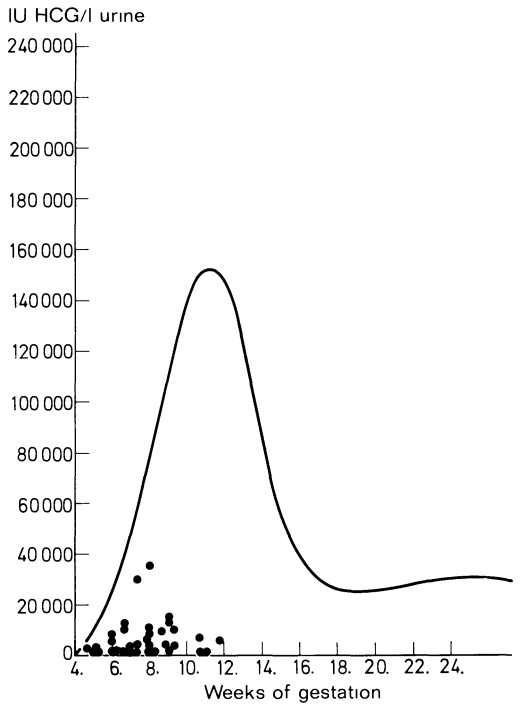


Fig. 13. Urinary HCG excretion in extra-uterine pregnancy (immuno-chemical assay). The curve corresponds to average normal excretion. (After KELLER, 1966)

3. Toxemias of Pregnancy

a) Hyperemesis Gravidarum

This condition is observed from the 2nd to 4th months of pregnancy and is therefore also termed early toxemia. Vomiting, often independent of the time of day and intake of food, is the dominant feature. This can cause electrolytic and metabolic disorders with adynamia, dehydration, weight loss, and tendency to syncope. The etiology is not clear, although psychological factors certainly play a part.

There is some controversy about the extent of endocrine involvement. HCG values are generally slightly increased. Since hyperemesis also commonly occurs in cases of hydatidiform mole with excessive HCG production, a certain connection is conceivable. Corticosteroid production and excretion are reduced, but the adrenal cortex responds fully to ACTH (STAEMMLER, 1956). Because of this, relative central adrenocortical insufficiency used to be considered a pathogenic factor, but even today, no definite pronouncement can be made about which of these changes are merely of secondary nature.

Environmental changes and psychological support are often the primary measures of treatment and can be obtained by admission to hospital. Sedatives can be of additional help. Antiemetics can be useful, but a certain amount of caution is necessary as a rule during the period of organogenesis. It is important, therefore, to treat the metabolic disturbance with infusions of adequate amounts of fluid, saline, and glucose. Later, a nourishing, easily digested diet can be considered. There is still no proof of any benefit obtained from treatment with hormones such as ACTH and corticosteroids. With correct treatment, the prognosis for the further course of pregnancy can be considered favorable.

b) Toxemia in Late Pregnancy

Toxemia in late pregnancy, also termed as EPH-gestosis, represents an exceptionally complex condition. Details can be obtained from textbooks of obstetrics. Clinical symptoms primarily include hypertension, proteinuria and formation of edema. The frequency of toxemia varies between 3.6% and 24.3% according to classification and region (FRIEDBERG, 1967). The rate is higher in primigravidas, especially those who are much younger or older than average, in multiple pregnancies, and in cases of hydatidiform mole. In severe cases, the features are characterized by tonic-clonic convulsions that progress into eclampsia, which is potentially fatal to mother and child.

There is still some controversy about the pathogenesis. Toxic, anaphylactic, endocrine, and metabolic factors have been considered. It is now generally assumed that uteroplacental ischemia may be involved. Animal experiments (OGDEN, 1940; BERGER, 1963) allow the conclusion that hypertensive substances may be liberated from the placenta in a manner reminiscent of the Goldblatt mechanism in the kidneys. It is also conceivable that this may cause constriction of the renal vessels with the result that angiotensin is released, causing a further rise in blood pressure. Adoption of any definitive attitude to these questions would be premature.

The primary pathophysiological finding is the generalized spasm of the arterioles, which leads to restriction of the circulation. Vascularization of the uterus and placenta may be considerably reduced, and the placenta may often show numerous infarcts of varying severity. In the kidneys, there are vascular constrictions and blocks in the region of the glomeruli. The glomerular filtrate may be greatly reduced, and

increased capillary permeability for plasma proteins may lead to proteinuria at the same time. Formation of edema is due to increased water and sodium retention in the interstitial space. The intravascular volume is diminished.

Endocrinological findings show that HCG values are discretely or quite markedly raised in the placenta (LORAINE, 1953), serum, and urine (LORAINE, 1950; VENNING, 1958). The renal clearance of this hormone can, however, be reduced in severe cases. The HPL level is normal in mild cases and often greatly reduced in severe cases (KELLER, 1970).

Estrogen values are reduced to varying degrees in the placenta, serum (RAY, 1963; RATANASAPA, 1967), and urine. In mild cases of pre-eclampsia, estriol excretion varies little from normal values, but in grave cases it falls considerably with no definite correlation between excretion and severity of the toxemia.

Placental progesterone is not greatly lowered (KUMAR, 1966), but its rate of production is not known. Normal or slightly reduced values are found in the plasma (SIMMER, 1959; COYLE, 1962). Pregnanediol excretion is only slightly pathologic in mild cases, but in contrast, may be grossly reduced in severe toxemia. The effect of restriction of the renal clearance has not been conclusively explained.

Aldosterone excretion is thought to be significantly lowered, possibly as a result of sodium retention. STARK (1963) gives an average value of 25 μg per day in comparison to 80 μg per day in healthy women at the end of pregnancy. The excretion of 17-hydroxycorticosteroids varies and allows no definite conclusions.

Dietary measures are the primary form of treatment of toxemia. Hypotensive drugs (Serpasil, Nepresol), sedatives (magnesium sulfate, barbiturates, phenothiazines), and diuretics (Hygroton, Lasix) can be used in severe cases. Details can be obtained from obstetrics textbooks.

The prognosis for severe toxemia is very poor when related to perinatal mortality. According to available statistics, fetal mortality is estimated at between 14 and 39% (FRIEDBERG, 1967) in eclampsia, and the mean maternal mortality between 4 and 6% (KYANK, 1963).

4. Rhesus Incompatibility

Rhesus immunization leads primarily to fetal damage. Placental changes can be considered secondary in nature. HCG values in the plasma and urine are occasionally found to be above normal limits. HPL titers have not so far been very characteristic. Estriol excretion is significantly lowered only in severe cases. Pregnanediol excretion is little changed, as is consistent with the findings above. Assessment of the fetal state is best done by means of spectral analysis of the amniotic fluid for the presence of biliary pigments.

Intra-uterine transfusions are now possible for the treatment of severe cases. Clinical improvement can be confirmed by a rise in estriol values in the urine (WYSS, 1970).

5. Multiple Pregnancies

The main result of a multiple pregnancy is an increase in the placental mass and total fetal weight. Production and excretion of HCG and HPL are correspondingly moderately raised. Elevated estriol (SCHWERS, 1968) and progesterone (SHORT, 1959) values are also found in the maternal plasma. Excretions of estriol and pregnanediol are generally within normal limits.

6. Prolongation of Pregnancy

The death rate in babies rises when pregnancy is prolonged by more than 10 days (MARTIUS, 1964). A higher incidence of placental infarction and impaired oxygen supply to the fetus can be observed. The causes of prolongation of pregnancy are obscure. In addition to individual variations, endocrine factors may also play some part, but the whole problem is still in a speculative phase (see p. 693).

According to ROSENKRANZ (1939), the estrogen and progesterone contents of the placenta are lowered in prolonged pregnancies. Plasma values are contradictory. Estriol and pregnanediol levels in the urine are normal or slightly reduced except in cases where there are manifest signs that pregnancy is impaired. Differentiation between true prolongation of pregnancy and biological postmaturity of the child is not possible in this way.

Supervision of a prolonged pregnancy is best done by physical means such as amnioscopy and cardiotocography rather than by diagnostic hormonal measures.

7. Fetal Malformations

Malformations, particulary anencephaly, are of special endocrinological interest. The fetal adrenal cortex shows pronounced hypoplasia in such cases. Growth is usually normal until the 20th week of pregnancy. Thereafter, the fetal zone undergoes atrophy or development comes to a stop. It is possible that HCG regulates the first phase of development, and that after

this, the hypothalamo-hypophyseal system is essential for normal development but is absent in the anencephalic fetus. It is not certain whether fetal LH (BENISCHKE, 1956) or ACTH (LANMAN, 1961) plays the crucial role.

The hypotrophic or atrophic adrenal cortex of these babies is unable to form the precursors necessary for the formation of estriol in the placenta (see p. 671), all of which must therefore be obtained from the maternal organism. It is thus not surprising that estriol excretion is usually extremely low. In contrast, placental function itself is more or less intact and progesterone production and pregnanediol excretion lie within normal limits (Fig. 14). The diagnosis can sometimes be suspected from this finding alone.

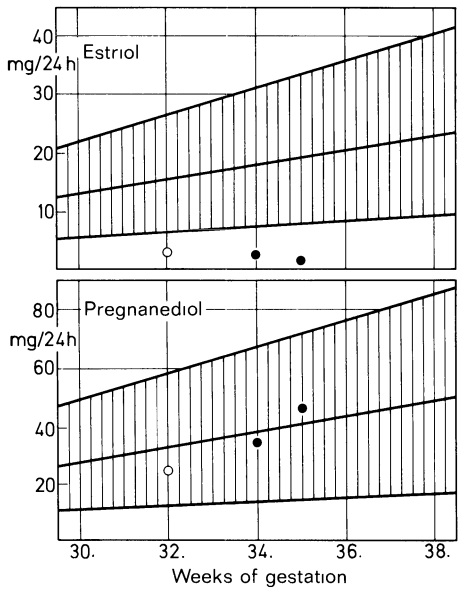


Fig. 14. Estriol and pregnanediol excretions in urine in anencephaly. The shaded zones correspond to normal 95% excretion limits. (After WYSS, 1970)

Other malformations usually produce no definite hormonal displacements. Estriol excretion can, however, occasionally be reduced in mongolism (MICHIE, 1967; WYSS, 1970), but it is not known whether hypoplasia of the adrenal cortex is involved.

8. Hydramnios

Placental function is usually not disturbed in hydramnios. Significant hormonal changes are found only in association with pathogenic anencephaly, diabetes, or multiple pregnancy.

9. Intra-Uterine Fetal Death

The placenta can sometimes maintain its endocrine function for a certain length of time after intra-uterine death of the fetus. Because of this, hormone values generally fall significantly more slowly than during the puerperium. Estriol excretion first falls within roughly 3 days to values below 200 μg/24 h. It then remains constant for some time since the placenta continues to receive estrogen precursors from the maternal organism, although in inadequate amounts. The mother probably also takes over the 16-hydroxylation required for placental formation of estriol.

Progesterone production can remain normal for days or even weeks, provided, of course, that there is no primary damage to the placenta. As a result, pregnanediol excretion in the urine also falls slowly. The same is applicable to the production and excretion of HCG and probably also HPL.

10. Hydatidiform Mole and Chorionepithelioma

a) Hydatidiform Mole

The hydatidiform mole is a benign malformation of the placental villi, which become distended in a characteristic manner (Fig. 15). A fetus is usually completely absent. It is a rare complication and an average frequency of 0.005–3.7% of all pregnancies has been calculated (HÖRMANN, 1965). Geographical distribution is important. For example, the hydatidiform mole is considerably more common in the Far East than in Central Europe.

Proliferation of the syncytioblast and cytotrophoblast in particular is a striking histological finding (Fig. 16). The stroma is swollen and edematous and contains few cells and vessels. Usually the whole placenta is involved in this change, but occasionally only individual sections are affected.

There is controversy about the pathogenesis of the hydatidiform mole. Developmental disturbances of the villous vessels (HÖRMANN, 1958), premature loss of the embryonal circulation, infections, etc. have been considered.

The hydatidiform mole usually becomes clinically manifest in the 3rd or 4th month. Often, a watery-sanguinous discharge is the first symptom and clear vesicles can occasionally be found in the discharge, confirming the diagnosis. The uterus is exceptionally large for the duration of amenorrhea. It is often of a doughy consistency. Heart sounds are absent and no fetal parts can be palpated.

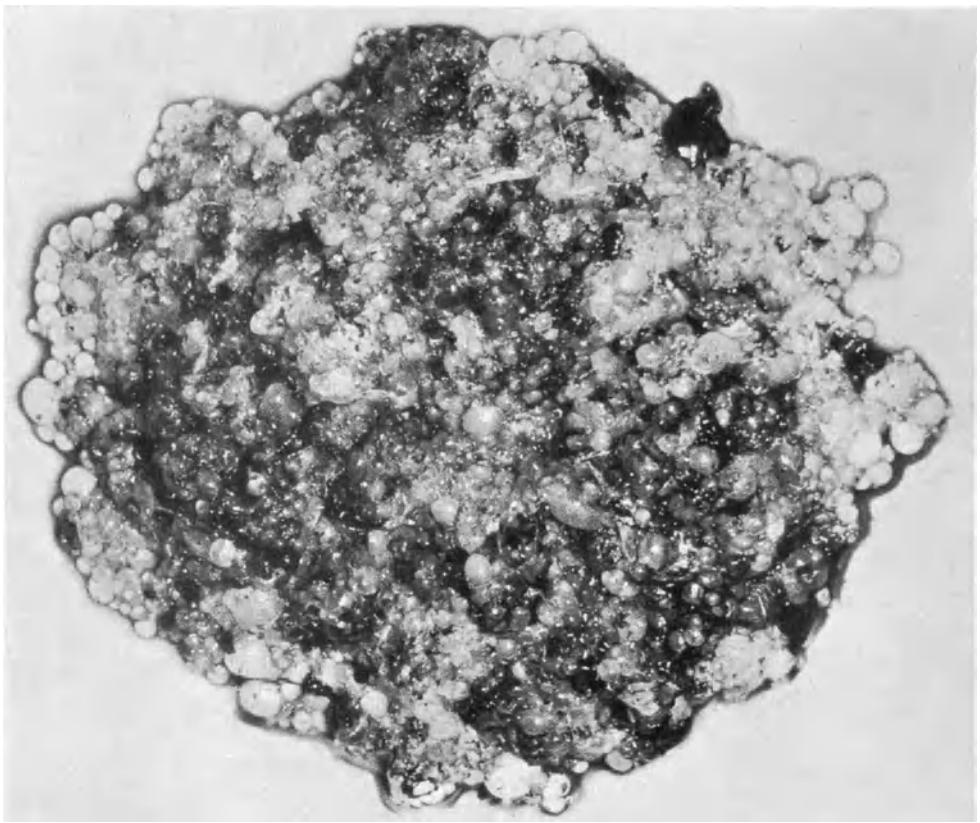


Fig. 15. Hydatidiform mole after expulsion

The hydatidiform mole is hormonally active and forms predominantly chorionic gonadotropin. The HCG titer is therefore greatly elevated in the mole itself as well as in the serum and urine (Fig. 17). The differences between the physiological excretion maxima are not sufficient to make a diagnosis, but values of 200000 to 2000000 IU HCG/l urine are evidence of a mole when pregnancy has reached the fourth month or beyond. Lower values, however, do not exclude a mole.

Production and secretion of HPL are usually greatly reduced in comparison to values found in a normal pregnancy of the same duration. The counteracting behavior of these two protein hormones can support the diagnosis.

The hydatidiform mole is probably capable of producing the same amounts of estrogens and progesterone as the normal placenta. However, estriol values in the urine are markedly reduced due to the absence of a fetus and thus an adequate supply of estrogen precursors to the placenta (FRANDSEN, 1964). Pregnanediol excretion lies within normal limits or is slightly lowered (STITCH, 1966; KAISER, 1953).

Probably due to the enormous production of HCG, bilateral lutein cysts, sometimes very large, are found in about 10% of cases. These cysts can also produce estrogens and progesterone and regress spontaneously after removal of the mole.

Treatment consists in surgical removal of the hydatidiform mole. This must be done very carefully and thoroughly. Frequent follow-ups with pregnancy tests are absolutely essential to avoid overlooking malignant degeneration into a chorionepithelioma. When the hydatidiform mole is completely removed, a negative result can be expected after two weeks at the latest. Otherwise at least semi-quantitative HCG estimations are necessary (see p. 699). The titer should continue to fall and any rise necessitates immediate thorough investigation.

b) Chorionepithelioma

The chorionepithelioma arises from malignant degeneration of the chorionic epithelium. It is very malignant, infiltrating locally and extending destructively. It metastasizes by way of the blood stream into the lungs and vagina. Microscopic examination shows proliferations of partly atypical chorionic epithelial cells of the

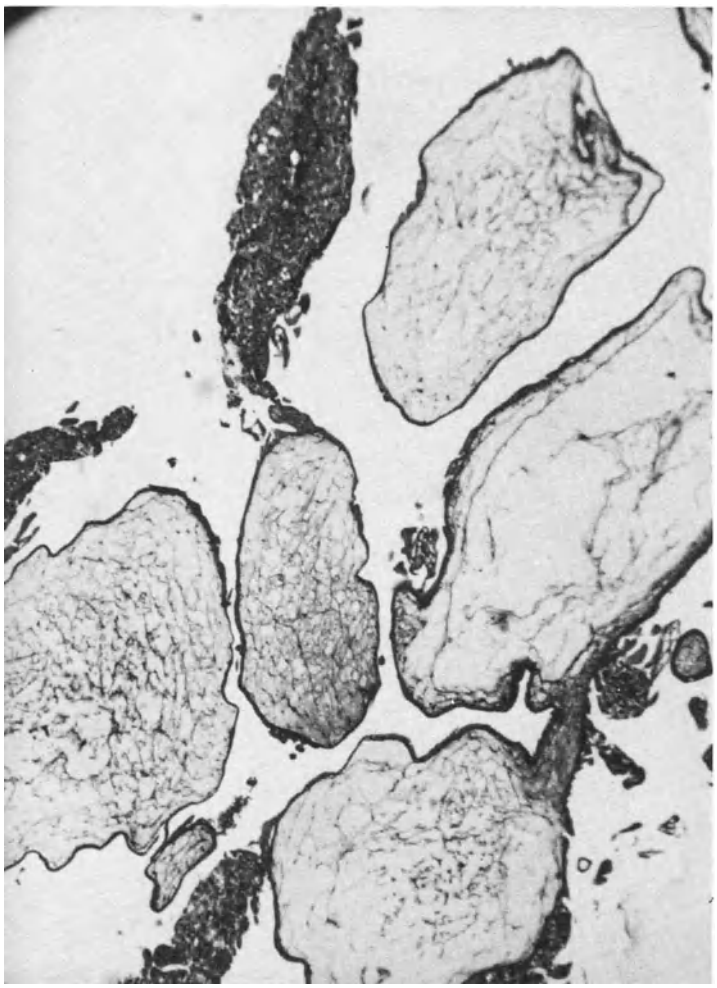


Fig. 16. Histological aspect of a hydatidiform mole

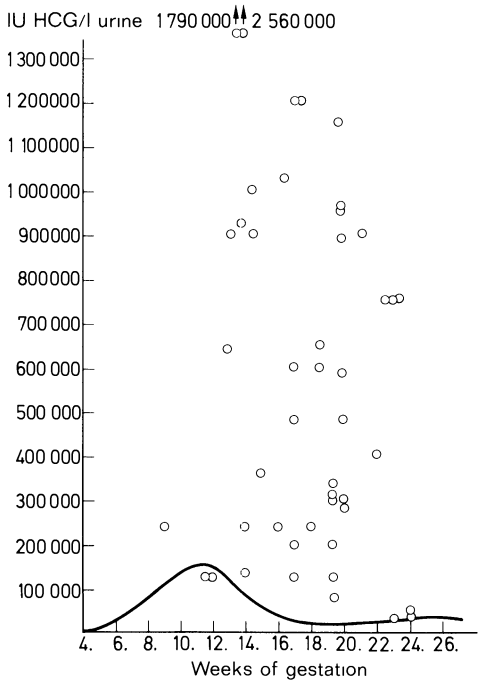


Fig. 17. Urinary excretion of HCG in hydatidiform mole (immuno-chemical assay). The curve corresponds to average normal excretion. (After KELLER, 1966)

syncytioblast and cytotrophoblast (Fig. 18). A more benign form, the chorionepitheliosis is thought to be distinguishable from malignant chorionepithelioma or choriocarcinoma (SCHOPPER, 1949) as the metastases present are not autonomous. Diagnosis by histological examination requires great experience.

The chorionepithelioma develops subsequent to a hydatidiform mole or abortion in about 40% of cases. In 20% it develops following a normal pregnancy (NOVAL, 1954). The latent period can be as long as 10 years. The frequency is one case in 8000–40000 pregnancies. There are strong regional variations, and like the hydatidiform mole, the chorionepithelioma is especially common in the Far East.

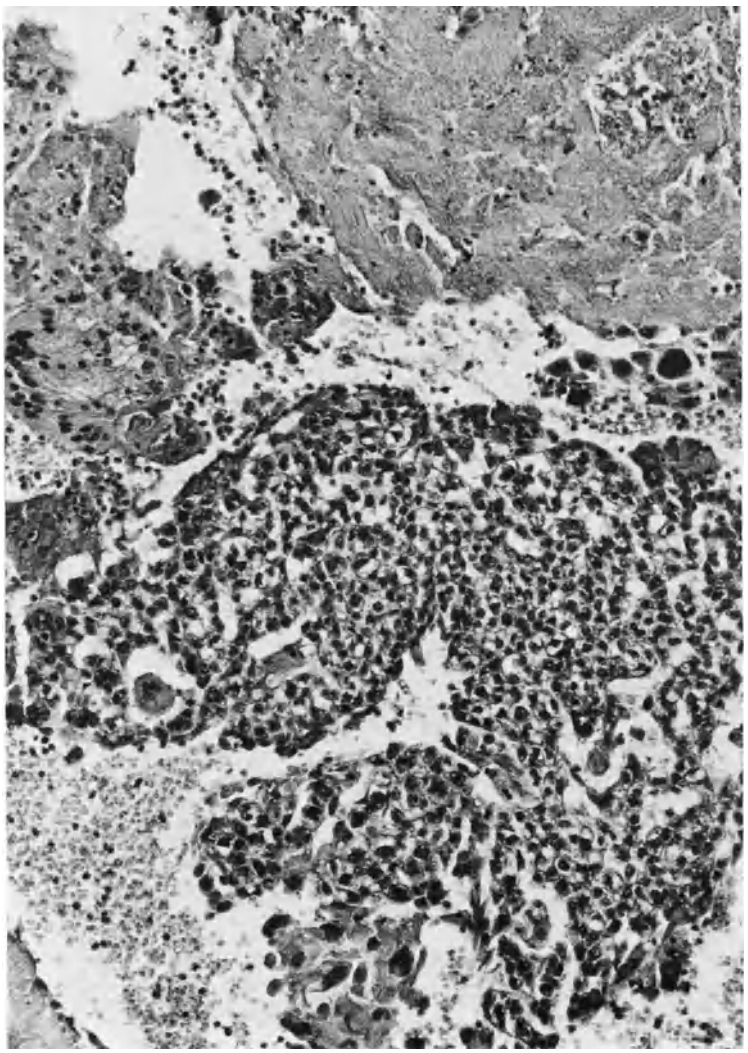


Fig. 18. Histological aspect of the chorionepithelioma

The hormone production is similar to that found with the hydatidiform mole. The formation of HCG, however, varies according to the differentiation of the tumor. Urinary excretory values are occasionally at the lower limits of sensitivity of the usual pregnancy tests commercially available. These values may, however, reach titers of 1 000 000 IU/liter. As in the case of hydatidiform mole, lutein cysts may also be observed.

Depending on the type, the chorionepithelioma is probably also capable of producing estrogens and progesterone. The irregular rise in the excretion of sexual steroids could, however, also be due to strong or mild stimulation of the ovaries by HCG.

Treatment lies mainly in the hands of specialists in oncology. In addition to surgical treatment, drugs, in particular aminopterin (Methotrexate), possibly in combination with other cystostatics (Leukeran, D-actinomycin), have recently been introduced into the treatment. HCG must be measured continually at each follow-up. Measurement of the excretion of estrogens and pregnanediol is of little practical value.

11. Hormonal Control of the Endangered Pregnancy

A short summary of the possibilities and indications of the usual present-day hormonal analyses used in monitoring an endangered pregnancy is presented below (Table 2).

Estimation of HCG is particularly suitable for controlling trophoblastic function in the

Table 2. Diagnostic significance of hormonal analysis in pathological pregnancies

	HCG	HPL	Estriol	Pregnanediol
Abortion	+++	+	−	−
Extra-uterine pregnancy	+++	−	−	−
Hyperemesis gravidarum	−	−	−	−
Toxemia in late pregnancy	−	+++	+++	++
Rhesus incompatibility	−	−	+	−
Plural pregnancy	−	−	−	−
Prolonged pregnancy	−	+	+	−
Fetal malformations	−	++	+++	++
Hydramnios	−	−	++	+
Intra-uterine fetal death	−	++	+++	++
Diabetes	−	+	+++	++
Hydatidiform mole	+++	+++	−	−

first trimester of pregnancy. HCG values are normal or only slightly reduced in cases of threatened abortion with a good prognosis. Greatly reduced values are generally found in cases with a poor prognosis. Low HCG titers are also characteristic of extra-uterine pregnancies. Values found in cases of hydatidiform mole and chorionepithelioma are usually slightly or excessively increased in comparison to those observed during a normal pregnancy.

HPL levels are low in cases of abortion where trophoblastic function is already impaired, in severe toxemia of pregnancy, and particularly with the hydatidiform mole. HPL is probably a measurement of almost pure placental function.

Excretion of estriol reflects placental as well as fetal function and is therefore especially suitable for early detection of fetal danger during the second half of pregnancy. Lowered values can be found as an expression of limited fetal production of precursors of estrogens in toxemia, diabetes, rhesus incompatibility, fetal malformations, and hydatidiform mole. Since individual values vary widely, estriol excretion must be measured regularly in endangered cases in order to recognize a fall as soon as possible.

Pregnanediol values again represent almost only placental function. The conclusive value is generally less than that obtained by measuring estriol; indications are roughly the same for both. In rare cases, however, placental insufficiency can first present as a reduction of pregnanediol excretion. On the other hand, the discrepancy between normal pregnanediol levels and low estriol levels in the presence of a living fetus can occasionally lead to the diagnosis of anencephaly.

In order to compensate for the disadvantage of true statistical methods of estimation, functional tolerance tests of the feto-placental unit using estrogen precursors, in particular dehydroepiandrosterone sulfate (DHA-S), have been suggested (Lauritzen, 1969; Van der Crabben, 1970). In the presence of intact function, intravenous administration to the mother results in 16-hydroxylation in the fetus with transformation into estriol in the placenta. As early as two hours afterwards, increased amounts of estriol are found in the plasma and urine. Important prognostic conclusions should be possible from the latent period and the degree of the rise. According to our experience, no binding conclusions can yet be made on this basis (Keller, 1970). In addition to hormonal diagnosis, amnioscopy, microanalysis of fetal blood, apparative registration of cardiac frequency, and analysis of the amniotic fluid now occupy a commanding position in supervision of the endangered pregnancy.

F. Hormonal Control of Birth

The causes of onset of labor have always been subject to numerous speculations. Spontaneous activity of the myometrium is directly influenced by four factors according to present-day knowledge – volume of the uterus, oxytocin, progesterone and estrogens. It must therefore be assumed that endocrine factors play a decisive role in the mechanism inducing birth. Details continue to remain uncertain for the time being, particularly in the human, and the whole concept is merely hypothetical (Fig. 19).

1. Steroid Hormones

There is particular controversy about the influence of estrogens on myometrial activity. In rats, ovariectomy results in considerable impairment of the process of birth or even complete arrest, while substitution with estrogens restores the normal process (Csapo, 1969). This suggests that estrogen deficiency would reduce uterine excitability and response. On the other hand, high doses of estrogens lead to premature birth in the same experimental animal (Csapo, 1969). The nervous response is also decreased in rabbits (Marshall, 1969). At the same time

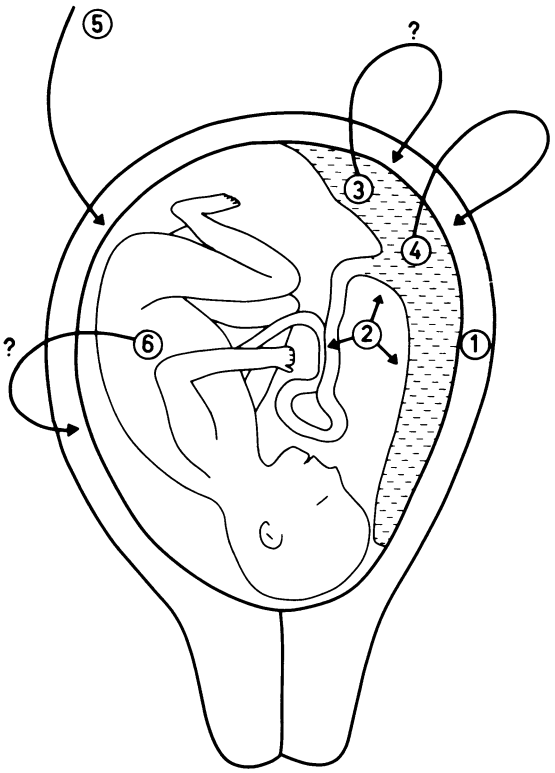


Fig. 19. Schematic representation of a few factors controlling induction of labor. *1* increase of myometrial activity, *2* internal uterine volume, *3* estrogens, *4* progesterone, *5* oxytocin, *6* fetal adrenal cortex

the membrane potential of the stimulation apparatus rises due to the increase in intracellular potassium ions.

The situation is more complex in the human. After intra-uterine death of the fetus associated with a massive fall in estrogen levels, labor does occur spontaneously after a varying interval, and contractions can also be induced without estrogen substitution. TURNBULL's findings (1967) are of interest. High estriol values and low estrone levels in the urine during the 34th week of pregnancy are claimed to be associated with early onset of labor, and low estriol levels and high estrone levels in the urine with prolonged pregnancies. According to these findings, estrogens may very well have a certain, but not necessarily essential influence on induction of labor.

More is known about the action of gestagenic hormones. In numerous animal experiments, progesterone suppressed neural excitability and uterine response to oxytocin, but this does not appear to be the case in guinea pigs (PORTER, 1969). In the woman, this blocking effect not only exists during pregnancy but even during the second half of the menstrual cycle. This is probably due to a displacement of the ionic distribution in the myometrial cells, with a shift of Ca ions in favor of Mg ions (COUTINHO, 1962).

These observations suggest that withdrawal of progesterone induces labor. In fact, PULKKINEN (1969) was able to show that the progesterone level fell in the blood before the onset of uterine contractions in legal abortion induced by intra-amniotic injection of hypertonic saline. Low values are also often found in spontaneous abortion. However, there is still no proof of a fall in progesterone levels prior to the onset of normal labor. CSAPO (1969) assumes that there is probably a state of equilibrium throughout pregnancy between the labor-promoting increase in uterine volume and the suppressive influence of the rising progesterone level, and that labor starts at term or prematurely when the ratio rises above a critical value.

2. Oxytocin

There is no doubt whatever that oxytocin can induce uterine contractions in women and animals. This hormone alone is not, however, the only factor responsible for induction of labor. This statement is supported by the facts that physiological doses of oxytocin do not induce labor during normal pregnancy, and that hypophysectomized women can have a normal labor.

During labor, the oxytocin titer in the plasma rises. This titer lies between 20–40 μ U/ml at the beginning of dilatation, which is no higher than in the non-pregnant state. When the cervix is dilated to about 5 cm, peak values of 2–4000 μU/ml are rapidly attained, and just as rapid a fall occurs within one hour after delivery (COUTINHO, 1962). Oxytocin must primarily be considered as a regulator of spontaneous uterine activity and not as a true inducer. Its action is dependent on adequate hormonal preparation. This can be demonstrated particularly well on the uterus of the menopausal woman, which responds to oxytocin only after appropriate preparation with sexual steroids. As mentioned above, progesterone and its influence on intracellular ionic relations are of primary importance.

The therapeutic value of oxytocin in the induction of labor and for weak contractions is of course not limited by these considerations.

3. Fetal Influences

From experiments with animals it has been recognized that corticosteroids can induce labor towards the end of pregnancy, particularly in cows and sheep (JÖCHLE, 1969). It is therefore

conceivable that the fetal adrenal cortex may have some effect in the induction of labor. In fact, extreme hypoplasia of the adrenal cortex results in prolongation of pregnancy in the cow. The same effect can be achieved in sheep by destruction of the hypophysis. As long as adrenalectomy is not performed at the same time, labor can be induced by intrafetal injections of ACTH (LIGGINS, 1967). In contrast, adrenocortical hyperplasia in goats produces a tendency to habitual abortion.

It is not known to what extent these findings can be applied to the human. Nevertheless, there is a tendency to prolonged pregnancy in anencephaly with extreme adrenal hypoplasia in the absence of hydramnios, whereas it has been found that the fetal adrenal cortex is markedly enlarged in unexplained premature births (TURNBULL, 1969).

G. The Postpartum Period

1. The Puerperium

With expulsion of the placenta the maternal organism loses the endocrine gland regulating pregnancy. Steroids as well as placental protein hormones disappear from the blood and urine very rapidly, as long as no placental tissue has been retained in the uterus.

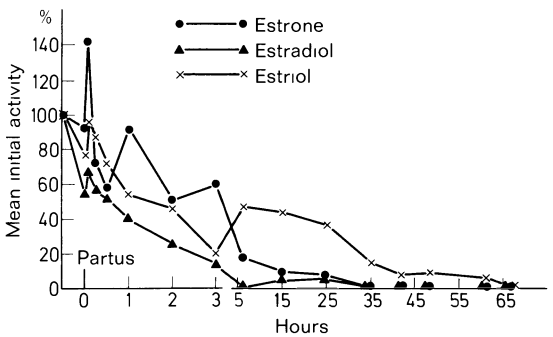


Fig. 20. Concentrations of estrone, estradiol and estriol in peripheral blood after expulsion of the placenta. (After ROY, 1963)

Estrogen levels in the plasma fall by 90% within one or two days (Fig. 20). Estradiol seems to disappear most quickly and is no longer measurable as early as 6 hours after the birth on average. Estrone takes 18–24 hours to disappear, and estriol about 42–60 hours (ROY, 1963). Urinary values of estradiol and estrone fall to normal non-pregnant limits within 2–3 days, and estriol excretion returns to normal within 7–8 days.

Progesterone also disappears from the maternal circulation within a few hours (Fig. 21), which is not astonishing in view of the extremely short half-life of about 5 min (SHORT, 1959). The level of the chief metabolite, pregnanediol, in the urine undergoes a steep fall during the first four postpartum days, followed by a more gradual decline. Non-pregnant normal values are reached within 6 days.

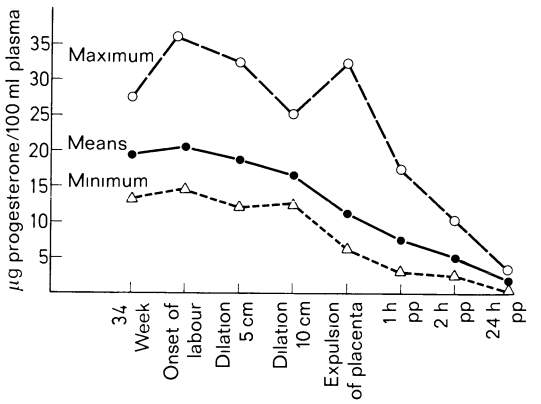


Fig. 21. Progesterone concentration in peripheral blood after expulsion of the placenta (gas chromatography). Average values and maximal deviation in 13 women. (After ZANDER, 1967)

The two most important placental protein hormones behave differently after birth. Plasma HCG falls by 50% within about 4 hours but small amounts are often still demonstrable after 10 days. Similar relations are found for urinary excretion, and the usual pregnancy tests are generally negative 1–2 days after, although they can occasionally remain positive for weeks.

In contrast, HPL disappears significantly more quickly, although hardly any is excreted in the urine and very high titers are present towards the end of pregnancy. This hormone is hardly demonstrable 3–4 hours after complete expulsion of the placenta (Fig. 22).

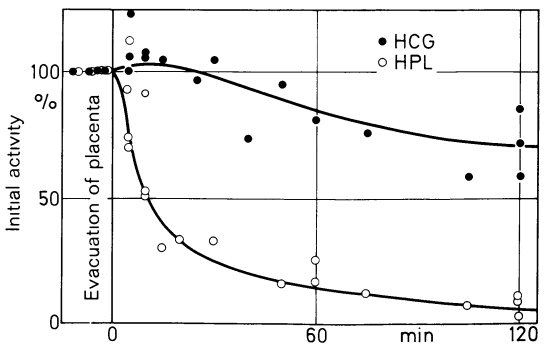


Fig. 22. HCG and HPL concentrations in the serum of 4 women after manual removal of the placenta (radioimmunoassay). (After KELLER, 1971)

Features of involution occur rapidly in parallel to these quick endocrine changes. The uterus regains its original form and size within 6 weeks, and after rejection of the decidua the normal endometrium regenerates within the same period of time. Changes in extragenital organs due to pregnancy also regress quickly.

2. Lactation

a) Anatomy and Physiology

The female mammary glands consist of 15–20 lobes separated from each other by connective tissue. Each lobe sends a lactiferous duct to the mamilla, where it dilates into the lactiferous sinus. The individual lobes are further subdivided into lobules which in turn consist of alveoli lined with *cylindrical* epithelium. In addition to the true glands, large amounts of adipose tissue are also present in the breasts. Smooth muscle is also found in the subareolar area and is responsible for erection of the nipples.

The breasts start to increase in size during the first trimester. The glandular tissue becomes more consistent, and the subcutaneous venous network and pigmentation become more marked. Microscopic findings are an increase and enlargement of the tubules and alveoli. There is no substantial secretion of milk but colostrum is formed.

After delivery, the so-called "rushing-in" of milk occurs during the first few days. The breasts become distended and are often tense due to intense hyperemia. All alveoli and discharging ducts are distended with milk. The normal mean daily production in both breasts together lies between 250 and 350 g one week after birth but can rise later to 1000 g.

During weaning, painful congestion is occasionally observed at first, but signs of involution soon appear. Numerous alveoli atrophy, so that a state almost identical to that prior to pregnancy is eventually achieved.

b) Hormonal Regulation

Lactation can be divided into four quite differently regulated phases; namely, mammogenesis (preparatory development of the breasts), lactogenesis (induction of milk secretion after delivery), galactopoiesis (maintenance of lactation), and finally, true ejection of milk during the act of suckling.

Hormonal preparation of the female breasts, *mammogenesis*, begins as early as puberty. The predisposed alveolar tissue develops under the influence of estrogens, and the lactiferous ducts begin to branch. HCG may also play a certain role at this stage of development. During pregnancy, the increasing amounts of estrogens produced lead to proliferation of the glandular ducts in particular. Progesterone results in further growth of the alveoli which continue to increase in number. HPL (see p. 670), corticosteroids, and thyroid hormones are probably also involved in the development (Fig. 23a).

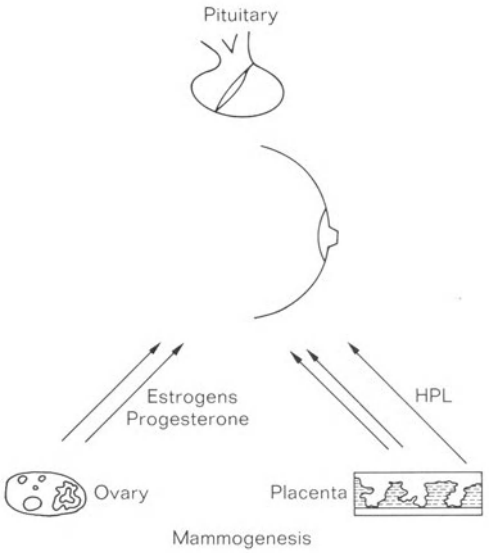


Fig. 23a. Hormonal regulation of mammogenesis

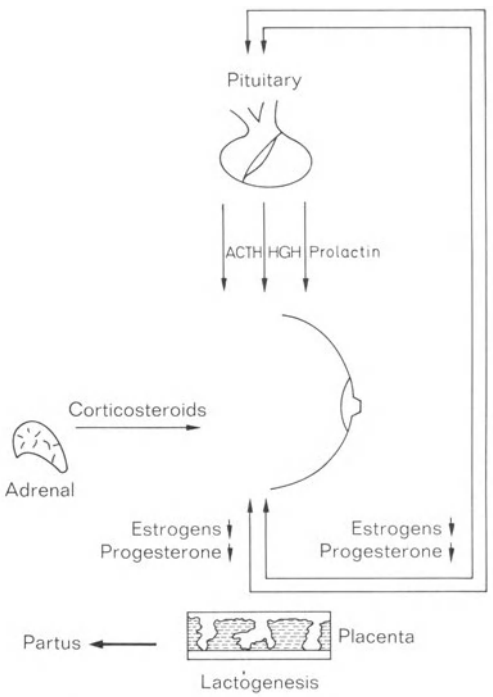


Fig. 23b. Hormonal regulation of lactogenesis

Induction of lactation, *lactogenesis*, is a very complex process (Fig. 23b). Loss of the high estrogenic and progesterone activity after expulsion of the placenta is a causal factor of primary importance. It is probable that during pregnancy these two hormones reduce the peripheral response of the alveoli to lactogenic factors and also inhibit the release of prolactin or other prolactin-like substances. Numerous experiments have indicated that prolactin certainly has a varying influence on lactogenesis, but its exact meaning in the human is still under investigation. Lactogenic, luteotropic and crop sac-stimulating properties, as well as the ability to act synergistically with estrogens to stimulate alveolar and tubular growth of the mammary glands, can also be attributed to purified HGH to an extent. Preparations with somewhat varying properties have been produced (see p. 674), and artefacts may be involved. However, there are other reasons to believe that prolactin and HGH exist in related but different forms in the human and that HGH at least is not essential for lactation. CANFIELD (1965) has shown that prolactin activity and HGH are increased during the phase of lactation and in galactorrhea in the absence of acromegaly. ROTH (1967) reported a marked rise in HGH activity and not in LTH activity after insulin-induced hypoglycemia. RIMOIN (1968) found that pregnancy and lactation were normal in cases of dwarfism with isolated HGH deficiency. PEAKE (1969) described a hypophyseal tumor in man, with LTH activity but no HGH activity. Lactogenesis is also dependent in some unexplained manner on corticosteroids and ACTH. Placental lactogen (see p. 670) is of little importance in this phase despite its prolactin-like action, because of its very rapid degeneration after expulsion of the placenta.

Galactopoiesis, the maintenance of milk secretion, is also dependent on various hormonal influences, particularly on prolactin, HGH, and TSH (Fig. 23c). Animal experiments have revealed that ACTH and corticosteroids are also essential (TALWALKER, 1961) and that corticosteroids probably also stimulate the formation of certain enzymes of the mammary glands. Estrogens and progesterone, on the other hand, have almost no effect in this phase. Ovarectomized women can breast-feed normally. Parathormone and insulin possibly also influence galactopoiesis but are not essential. Psychological factors, such as the stimulus from continual suckling, which probably influences prolactin secretion via neural paths, are of greater importance. Loss of these factors results very quickly in drying up of milk formation.

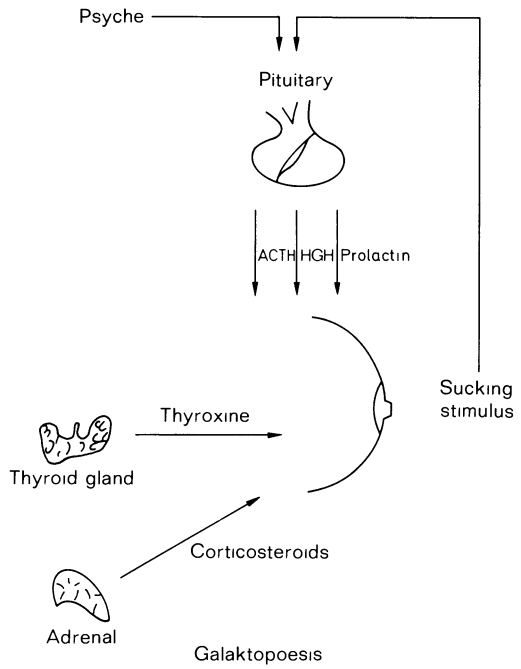


Fig. 23c. Hormonal regulation of galactopoiesis

True milk ejection during breast-feeding (Fig. 23d) is governed primarily by oxytocin which is released from the neurohypophysis through the suckling stimulus by means of a neurohumoral reflex arc. Oxytocin then causes contraction of the myoepithelial elements of the mammary glands (milk let-down effect). The necessary rise in intracanalicular pressure is mainly promoted by the chewing and sucking movements of the child. It is still not exactly

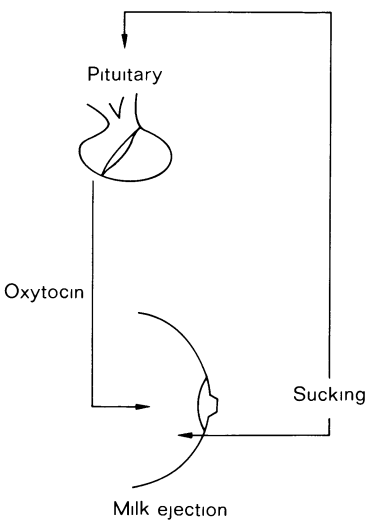


Fig. 23d. Hormonal regulation of milk ejection (Milk let down)

known whether oxytocin also exerts a central influence on prolactin release. Vasopressin may have effects vaguely similar to those of oxytocin.

c) Suppression of Lactation

When necessary, lactation can be suppressed by the administration of sexual steroids. Estrogens may be used alone or in combination with androgens or gestagens. Milk secretion already in existence can be inhibited in this manner, and induction of lactation can also be prevented if the hormones are used at the right time. Inhibition of prolactin secretion might have a considerable effect in suppressing lactation. The most potent lactation suppressing agent today with practically no side effects is 2-Br.-α-ergocryptine (FLÜCKIGER, 1968; BRUN DEL RE (in print)). It inhibits the release of prolactin from the anterior pituitary. 5 mg daily p.o. suffice to stop laction. (See also Chap. V, p. 83).

The following drugs also have proven satisfactory for the suppression of lactation before the onset of breast-feeding: ethinyl estradiol (Lynoral, Progynon M) in a dose of 0.2 mg for about 6 days, or diethyl stilbestrol (Oestremon) in a dose of 1 mg for 6 days. Estradiol dipropionate (Ovocyclin) in a dose of 10 mg can also be given intramuscularly for 2–3 days or 1 ampoule Ablacton (combination of 5 mg estradiol benzoate, 8 mg estradiol valerianate, 20 mg norethisterone acetate and 180 mg testosteronoenanthate) immediately after delivery.

Success is generally better, the earlier administration of drugs is started. Sometimes larger doses are needed in women who have already started to breast-feed, and individual response varies. An intramuscular injection of 10 mg Ovocyclin/day for 2–3 days or 1 ampoule of Ablacton can be recommended in these cases too. Supportive measures are restriction of fluid intake, supportive bandages for the breasts, cold compresses, laxatives and diuretics.

d) Pathologic Lactation

Deficient milk production (hypogalactia) or failure to produce milk (agalactia) may be due to anatomical and functional factors, such as hypoplasia of the glands, anomalies of the nipples, previous mastitis and weak suckling by the infant. Postpartum hypopituitarism (see p. 92) is the most common of the rare endocrine causes.

Hormonal treatment in deficient milk production is not very successful except in true disorders of milk output. In cases where the breasts are not very productive, oxytocin, for example in the form of an intranasal Syntocinon spray (4–5 IU) shortly before breast feeding, can be useful. Sublingual tablets are also available for the same purpose. The milk is expelled from the alveoli into the larger draining ducts under the influence of oxytocin, i.e. emptying is promoted. The amount of milk is thought to be increased at the same time.

Persistent lactation after weaning, as is seen in combination with amenorrhea and genital hypoplasia in the Chiari-Frommel syndrome (see p. 602), is another endocrine disorder of milk production.

Puerperal mastitis, the most common complication during breast feeding, is of no endocrinological significance.

3. The Menstrual Cycle

a) Physiology

With the loss of excessive steroids produced by the placenta, the adenohypophysis resumes the secretion of normal amounts of gonadotropins from about the 10th day after birth (KELLER, 1968). The time at which the first menstruation occurs, however, is extremely variable and depends, among other factors, on lactation. According to SHARMAN (1956), 91%

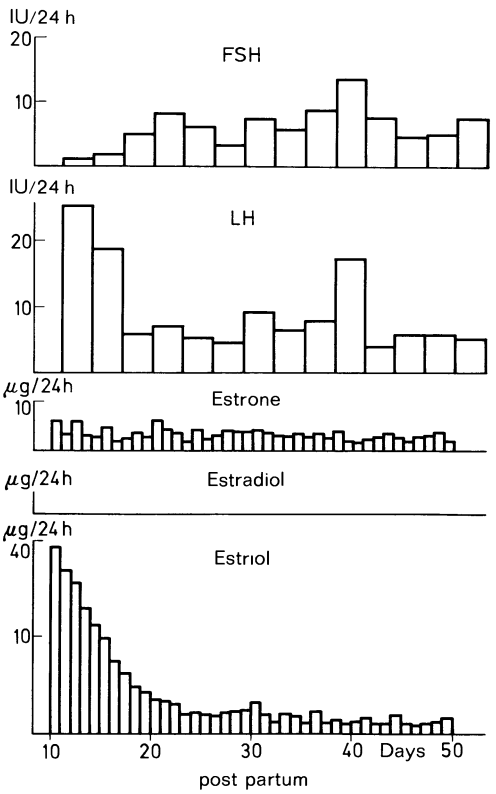


Fig. 24. Urinary excretion of FSH, LH and estrogens during lactation. (After original figures from individual cases of KELLER, 1968, and BROWN, 1956)

of women who do not breast-feed menstruate during the first three months after delivery, and only 33% of women who do breast-feed; the cycles are often anovulatory, especially when arising early. In the above investigations, it was found that the 42nd postpartum day was the earliest time at which ovulation occurred in the group of women not breast-feeding, and the 31st week in women who were breast-feeding.

The reasons for amenorrhea during lactation are not explained. The secretion of FSH and LH does not appear to differ much from that during the normal menstrual cycle, and a few LH peaks have even been observed (Fig. 24). In contrast, estrogen excretion remains very low after the puerperium and is still reduced even in the first ovulatory cycle (BROWN, 1956). From this it can be assumed that ovarian response to gonadotropic hormones is temporarily reduced.

There is no doubt that fertility is considerably reduced during the phase of lactation, but there is no absolute protection against conception since ovulation may occur occasionally during this time.

b) Pathology

Gonadotropic function of the anterior lobe of the hypophysis can occasionally be diminished for a long time following birth, so that a picture of secondary, usually hypogonadotropic amenorrhea arises. The prognosis is favorable on the whole.

Sheehan's syndrome due to postpartum hypopituitarism is a much more severe complication (see p. 91). It arises subsequent to delivery complicated by severe hemorrhage or collapse resulting in ischemic necroses of the adenohypophysis. Symptoms can vary widely, depending on the degree of destruction. In typical cases, gonadotropic as well as thyrotropic and adrenocorticotropic regulation are affected. Gonadotropic function seems to be the most sensitive. Agalactia, genital hypoplasia, amenorrhea, adynamia, pigmentation disturbances and loss of secondary sexual hair are early symptoms. Details of the later symptoms, diagnostic methods and therapy can be found on p. 93.

H. Endocrinological Methods of Investigations during Pregnancy

1. Pregnancy Reactions

Pregnancy reactions for the early diagnosis of pregnancy are now based almost exclusively on the qualitative detection of HCG. Special discussion is necessary since these tests, which can now be performed by any doctor in his own laboratory, offer considerable practical advantages over the quantitative methods of determination in technical simplicity and stability, reliability, and most of all the amount of time involved.

a) Biological Methods

Until recent years, only animal tests were available, and numerous different reactions were recommended during the course of time. The Ascheim-Zondek test proved particularly suitable. In this test the urine under investigation is filtered and injected into infantile mice at regular intervals. The result is positive if follicles develop after four days; these follicles show hemorrhages, which look like spots of blood, and corpora lutea. The hyperemic reaction in rats occurs more quickly (KUPPERMAN *et al.*, 1943). The urine is best administered intraperitoneally in infantile animals, and an obvious hyperemia can be seen in the ovaries as early as a few hours after injection in a positive case. The Friedman reaction in adult female rabbits is based on a similar principle. The urine is injected in the vein of the ear, and if adequate amounts of HCG are present, hemorrhagic follicles and corpora lutea develop 48 hours later. The animals can be used for further pregnancy reactions after the laparatomy.

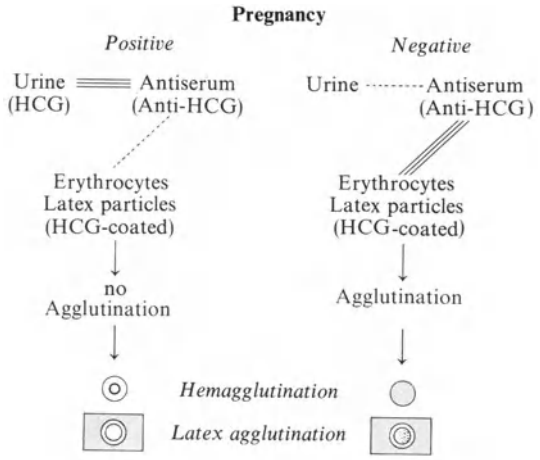
Finally, the release of spermatozoa by male amphibia can also be used as a pregnancy test, as in the Galli-Mainini reaction in sexually mature toads or frogs. The urine is injected into the dorsal lymph sacs of the animals. Then, urinary specimens are taken at short intervals from the cloaca and microscopically examined. Spermatozoa are found in positive cases.

All the reactions mentioned are highly reliable but most have been completely abandoned today in favor of the immunochemical pregnancy tests because of the costly expenditure of time and the problem of keeping the animals.

b) Immunological Methods

The agglutination-inhibition tests, based principally on WIDE's work (1960), are most frequently considered for practical purposes. The reaction involved is an immunochemical reaction between tanned sheep erythrocytes coated with HCG and a specific antiserum against HCG. Agglutination normally arises but is inhibited if HCG, for example in the form of pregnant urine, is added to the system. This results in binding of the antiserum available (Scheme 4).

Scheme 4. Principle of immunological pregnancy reactions



Latex particles coated with HCG can also be used instead of erythrocytes.

The Pregnosticon Test (N. V. Organon) is the most extensively used hemagglutination test and is commercially available. The ampoules contain HCG-coated erythrocytes, antiserum and the buffer substance in lyophylized form for this procedure. The test is performed as follows: an ampoule is opened, 0.1 ml filtrated urine from a morning specimen and 0.4 ml distilled water are added. The ampoule is shaken for a short while and put into a stand with a mirror (Fig. 25). The result is read after the system has been allowed to stand undisturbed for two hours. Inhibition of agglutination, i.e., a positive reaction, is expressed by the development of a marked red-brown sedimentation ring at the bottom of the ampoule. Agglutination is depicted by a diffuse carpet of erythrocytes (negative reaction) (Fig. 26). The sensitivity is about 1000 IU HCG/l, and positive results can be expected on average after the 22nd to 24th day following conception. The degree of accuracy is extremely high, but false-positive patterns can be observed occasionally if urine or glassware are contaminated with detergents or soap, or in cases where the luteinizing hormone is excreted in abnormally high amounts and cross-reacts with the HCG, as, for example, in certain endocrinopathies, early in the climacteric or at mid-cycle. False-negative results only occur with very low HCG values as in extra-uterine pregnancies and during the very early stages of pregnancy.

The latex-agglutination inhibition tests, such as Gravindex (Ortho) or Pregnosticon-Planotest

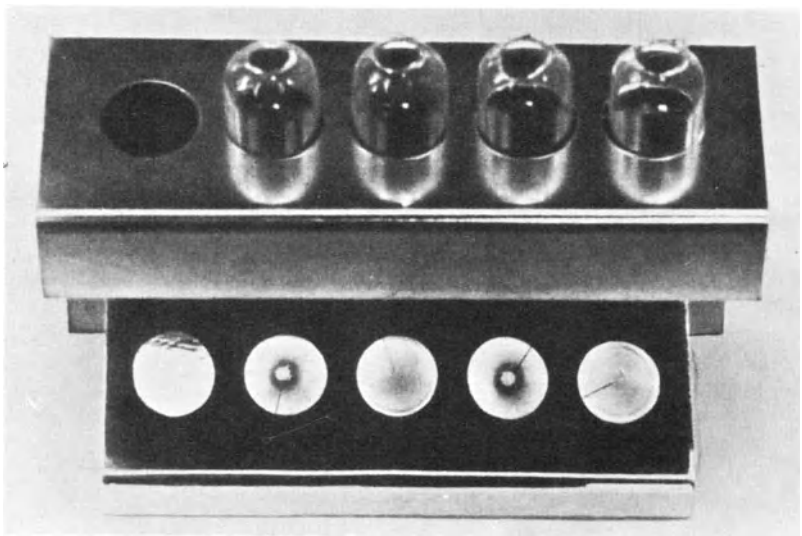


Fig. 25. Pregnosticon test. Stand with mirror and inserted ampoules

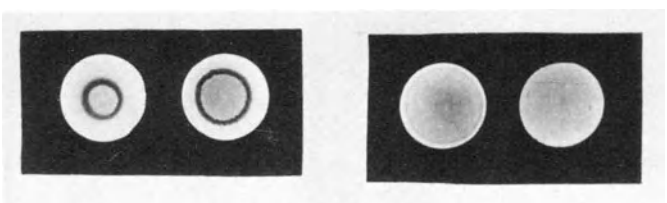


Fig. 26. Positive (left) and negative (right) hemagglutination test (Pregnosticon)

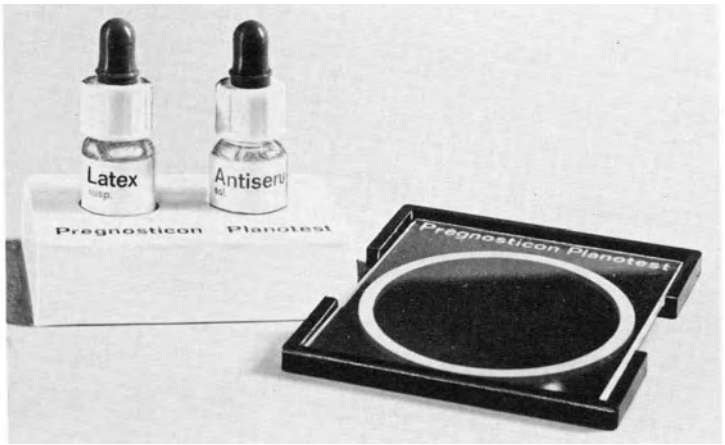


Fig. 27. Pregnosticon-Planotest. Microscope slide, small bottles with latex suspension and anti-serum

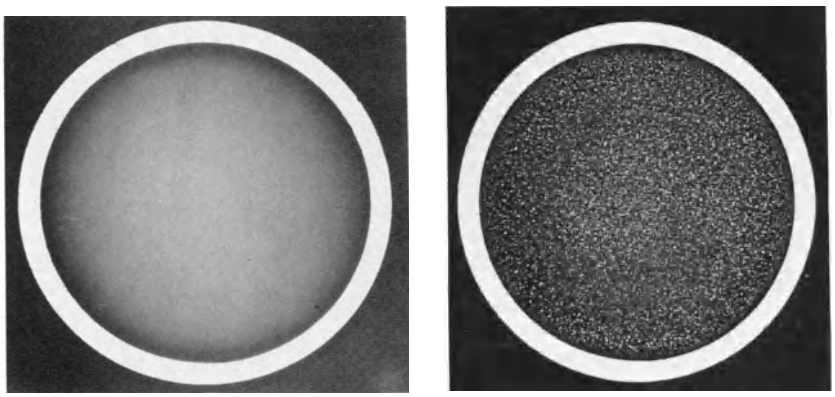


Fig. 28. Positive (left) and negative (right) latex agglutination test (Pregnosticon-Planotest)

(N. V. Organon), take even less time. In this procedure (Fig. 27) a drop of morning urine and a drop of antiserum are mixed together for 30 sec on a microscope slide with a ring drawn on it. A drop of latex suspension is then added, and the mixture is spread out to fill the size of the ring by means of a small plastic rod. The slide is tilted back and forth for 2 min to mix the substances. In a positive case agglutination is inhibited and the suspension remains milky and cloudy. In a negative case, agglutination of the latex particles occurs (Fig. 28). The sensitivity is about 3000 IU HCG/l, and positive results can be expected 24–26 days after conception. The degree of accuracy is also very good except in cases with very low HCG values, as in extra-uterine pregnancies.

2. Quantitative HCG Estimation

a) Biological Method

HCG is measured quantitatively on the basis of reactions not dependent on steroid influences. Increase in weight of the whole prostate or the ventral lobe (LORAINE, 1950; APOSTOLAKIS, 1958), of the seminal vesicles or of all the accessory sexual glands (DICZFALUSY, 1954) in infantile male rats has proved most satisfactory. The ascorbic acid-depletion test has also given good results (PARLOW, 1959), whereas the large number of other biological methods recommended are inferior in precision, specificity, and sensitivity. This is also applicable to the semi-quantitative method of the classic pregnancy reactions by means of dilution series. The results should always be given in IU for which the first and second international standard preparations were established in 1939 and 1954 respectively.

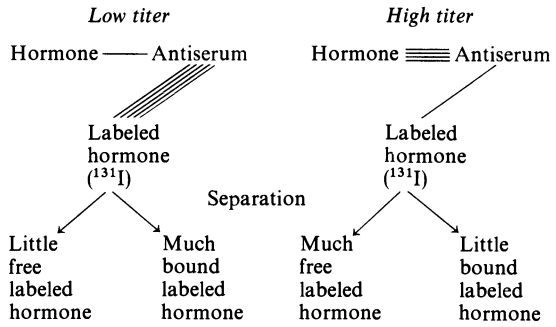
b) Immunological Methods

HCG in the urine can be measured semi-quantitatively with the hemagglutination and latex-agglutination tests described (see p. 697) by applying a corresponding dilution series to the urine under investigation. The titer searched for is calculated on the basis of the neutral point

of the last still positive dilution. This method is still of considerable importance in clinical work, for example, in diagnosing the hydatidiform mole.

A complement-fixation reaction for serum has been worked out by BRODY (1960), but it has aroused little practical interest. Chorionic gonadotropin is best and most reliably measured by radioimmunological methods. These procedures are based on competitive binding between HCG labeled with ^{131}I or ^{125}I and the HCG to be measured in the serum to a specific antiserum obtained from rabbits or guinea pigs. After an incubation period the bound fraction is separated from the free fraction by means of chromatoelectrophoresis, chemical, or immunological precipitation procedures, and the ratio can be determined by measurement of the radioactive impulses. If the HCG titer is low, then at a certain adjustment of the system, a large proportion of labeled HCG can be bound. If the titer is high, only a small amount of antiserum is still available for binding with the labeled hormone (Scheme 5).

Scheme 5. Principle of radioimmunological estimation of protein hormones



After an appropriate standard curve has been established, a very precise quantitative system can be constructed. This system is also extremely sensitive and is most suited for serum, whereas urine must be previously dialyzed and results obtained are less reliable.

3. Methods for Estimating HPL

a) Biological Methods

HPL cannot be uniformly tested biologically because of its various types of actions. The tibia test is the first test of importance and is also used for determining HGH, a closely related compound (see p. 123). Theoretically, the classical pigeon crop sac test (see p. 701) and the deciduoma test (see p. 701) can also be considered on the basis of the prolactin-like and luteotropic properties, but these methods are really of little use for the estimation of HPL.

b) Immunological Methods

In theory, HPL can be determined in the same manner as HCG; the method of choice is the radioimmunological technique (SAXENA, 1968). Separation of the bound and free fractions can easily be done by means of a charcoal-dextran system. Unfortunately, no international reference preparation is yet available, so that results must be given in equivalents of purified material.

4. Estimation of Estrogens

In theory, estrogens can be estimated by biological (see p. 547), chemical, and now also by immunological methods. For research purposes, numerous precise but costly and tedious methods are now available. The discussion below is limited to practical important methods for use with urine during pregnancy.

Estrogens can be measured as a whole; the methods after JAYLE (1959) and ITTRICH (1958) are particularly suitable for this purpose. JAYLE'S method entails enzymatic hydrolysis, conversion of the estrogens into their methyl ether, and colorimetric estimation by means of the Kober reaction. ITTRICH'S method involves extracting the Kober chromogens with p-nitrophenol and then measuring them by fluorimetric means.

For practical purposes the shorter modification after BROWN (1963) has also proved suitable. The urine is hydrolyzed with acid, extracted with ether and sodium hydroxide and incubated with dimethyl sulfate. Further purification is achieved by oxidation with $KMnO_4$ and extraction with benzol. The Kober reaction is then carried out directly with sulfuric acid and hydroquinone and colorimetrized at 465, 515 and 565 nm. The procedure does not necessitate any special apparatus and is very reliable for measuring high concentrations of estriol. With some modifications and the use of an extractor, the method can be done semiautomatically (BROWN, 1968). If the necessary apparatus is available, estriol can also be measured quickly and reliably by means of gas chromatography.

5. Estimation of Pregnanediol

In addition to estrogens, progesterone is also of practical interest during pregnancy. Determination of serum progesterone is for the time being restricted to experienced centers, so that

routine investigation is usually limited to the measurement of pregnanediol, the main metabolite excreted. In addition to gas chromatographic methods, the method of KLOPPER (1958) has proved particularly suitable for routine purposes. It involves hydrolysis with hydrochloric acid, extraction with toluene, purification of the total extract with NaOH and $KMnO_4$, primary chromatography on aluminium oxide with ethyl alcohol and benzol, acetylization and further purification with sodium bicarbonate, followed by a second chromatography of pregnanediol acetate on aluminium oxide with benzol. Colorimetry occurs at 425 nm. The method is highly reliable and specific although somewhat time-consuming.

6. DHAS Test

In this procedure, dehydroepiandrosterone sulfate (DHAS) is given to the mother intravenously to test the state of the fetoplacental unit. Estriol is measured in the urine or plasma before and after administration of this compound. Details of the procedure are still insufficiently worked out and DHAS is not available for general use in a suitable form. The results are also contradictory so that a detailed presentation is not appropriate at this point.

7. Estimation of Prolactin

The proliferation test on the pigeon's crop sac is the method most extensively used for the demonstration of prolactin activity. Originally, the material was administered to the animal intramuscularly and the increase in weight of both crop sacs served as the end point. Intradermal application is now generally prefered because of the higher sensitivity. Nonspecific inflammatory effects are prevented by giving prednisone at the same time. The proliferative zones can either be measured, histologically assessed, or excised and weighed.

Another possibility is to test the lactogenic effect on the mammary glands of rabbits. The material is introduced into the duct of the teat. A similar principle is involved in the measurement of the *in-vitro* lactogenic effect on tissue cultures or sections of mammary glands of mice. This method is highly sensitive.

The luteotropic activity of prolactin can either be determined by means of the formation of a deciduoma in intact and hypophysectomized mice or by means of the hyperemic effect in the corpus luteum (KOVACIC, 1963). Pregnanediol excretion in hypophysectomized, adrenalectomized rats has also been used to assess luteotropic activity (BLOBEL, 1966).

A very recent development is the successful application of immunological methods to the measurement of prolactin. Radioimmunological methods are again of primary interest (JACOBS, 1972).

Results in the human, however, are still controversial.

References

Anatomy

BARTELS, H., MOLL, W.: Physiology of gas exchange in the human placenta. Amer. J. Obstet. Gynec. **84**, 1714 (1962).

BROWNE, J.C., MCCLURE: Utero-placental circulation. Cold Spr. Harb. Symp. quant. Biol. **19**, 60 (1954).

ELERT, R.: Hypophyse. In: SEITZ, L., AMREICH, A.I., Biologie und Pathologie des Weibes, Bd. 1, Teil 1, S. 473. München: Urban & Schwarzenberg 1953.

GREENFIELD, A.D.M., SHEPPERD, I.T., WHELAN, R.F.: The rate of blood flow in the umbilical cord. Lancet II, 422 (1951).

NELSON, W.W., GREENE, R.R.: The human ovary in pregnancy. Int. Abstr. Surg. **97**, 1 (1953).

ROMNEY, S.L., DUNCAN, E., REID, M.D., METCALF, J., BURWELL, S.: Oxygen utilization by the human fetus in utero. Amer. J. Obstet. Gynec. **70**, 791 (1955).

SCHREINER, W.E.: Die plazentaren Funktionen und ihre Störungen. Gynaecologia (Basel) **161**, 372 (1966).

SNOECK, J.: Le placenta humain. Paris: Masson 1958.

STRAUSS, F.: Bau und Funktion der menschlichen Plazenta. Fortschr. Geburtsh. Gynäk. **17**, 33 (1964).

WILKIN, P.: Pathologie du placenta. Paris: Masson 1965.

Chemistry and Biochemistry of Hormones Regulating Pregnancy and Lactation

Placental Proteohormones

WOLSTENHOLME, G.E.W., KNIGHT, J. (eds.): Lactogenic hormones. Edinburgh and London: Churchill Livingstone 1972.

BECK, P., DAUGHADAY, W.: Human placental lactogen: Studies on its acute metabolic effects and disposition in normal man. J. clin. Invest. **46**, 103 (1967).

BLOBEL, R.: Über das Choriongonadotropin. Fortschr. Geburtsh. Gynäk., Bd. 40. Basel: Karger 1966.

— UHLIG, H., SCHUHMACHER, G.: Biochemical studies with human chorionic gonadotropin (HCG). Acta endocr. (Kbh.) **67**, 72 (1962).

CATT, K., MUFFAT, B.: Fractionation of rat pituitary extract by starch gel electrophoresis and identification of growth hormone and prolactin. Endocrinology **76**, 678 (1965).

FLORINI, J.R., TONELLI, G., BREUER, C.B., COPPOLA, J., RINGLER, I., BELL, P.H.: Characterization and biological effects of purified placental protein (human). Endocrinology **79**, 692 (1966).

FORSYTH, I.A.: Prolactin and placental lactogens. In: GRAY, C.H., BACHARACH, A.L., Hormones in blood, Bd. 1, 2. ed., S. 234. London and New York: Academic Press 1967.

GOT, R.: Gonadotrophine choriale humaine. Isolement et characterisation. Thèse de sciences, Soc. Saint-Quentinoise d'Imprimerie, Paris, 1959.

— BOURRILLON, R.: Nouvelles données physiques sur la gonadotropine choriale humaine. Biochim. biophys. Acta **42**, 505 (1960).

GRUMBACH, M.M., KAPLAN, S.L.: In vivo and in vitro evidence of the synthesis and secretion of chorionic "growth hormone prolactin" by the human placenta: Its purification, immunoassay and distinction from human pituitary growth hormone. In: Proc. 2. Int. Congr. Endocrin., S. 691. Amsterdam: Excerpta Medica 1965.

HELL, H. VAN, GOVERDE, B.C., SCHUURS, A.H.W.M., DE JAGER, E. MATTHIJSEN, R., HOMAN, J.A.H.: Purification, characterization and immunochemical properties of human chorionic gonadotropin. Nature (Lond.) **212**, 261 (1966).

HENNEN, G., PIERCE, J.G., FREYCHET, P.: Human chorionic thyrotropin: Further characterization and study of its secretion during pregnancy. J. clin. Endocr. **29**, 581 (1969).

JOHNSON, C.E., ALBERT, A., WILSON, R.B.: Renal and extrarenal disposal of chorionic gonadotropin in the immediate postpartum period. J. clin. Endocr. **10**, 371 (1950).

JOSIMOVICH, J.B., MACLAREN, J.A.: Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone. Endocrinology **71**, 209 (1962).

KAPLAN, S.L., GRUMBACH, M.M.: Serum chorionic "growth hormone prolactin" and serum pituitary growth hormone in mother and fetus at term. J. clin. Endocr. **25**, 1370 (1965).

KELLER, P.J., GERBER, C., GREUB, H., SCHREINER, W.E.: Studies on the metabolism of human placental lactogen in pregnancy. Horm. Metab. Res. **2**, 34 (1970).

LEEB, H., SCHNEIDER, W.: Probleme des Choriongonadotropinstoffwechsels bei der Ratte. Wien. klin. Wschr. 391 (1956).

LEZNOFF, A., DAVIS, B.A.: The cytological localization of human chorionic gonadotropin. Canad. J. Biochem. **41**, 2517 (1963).

LORAINE, J.A.: The renal clearance of chorionic gonadotrophin in normal and pathological pregnancy. Quart. J. exp. Physiol. **36**, 11 (1950).

— Human chorionic gonadotrophin. In: GRAY, C.H., BACHARACH, A.L., Hormones in blood, 2. ed., Bd. 1, S. 313. London and New York: Academic Press 1967.

MIDGLEY, A.R., JR., PIERCE, G.B., JR.: Immunohistochemical localization of human chorionic gonadotropin. J. exp. Med. **115**, 289 (1962).

NYDICK, M., BERRY, R.J., ADELL, W.D.: Molecular weight of human chorionic gonadotropin as estimated by means of radiation inactivation of biological activity. J. clin. Endocr. **24**, 1049 (1964).

PHILIPP, E.: Die Bildungsstätte des Hypophysenvorderlappenhormons in der Gradividität. Zbl. Gynäk. **54**, 450 (1930).

REISFELD, R.A., HERTZ, R.: Purification of chorionic gonadotropin from the urine of patients with trophoblastic tumors. Biochim. biophys. Acta **43**, 540 (1960).

SCIARRA, J.J., KAPLAN, S.L., GRUMBACH, M.M.: Localization of anti-human growth hormone serum within the human placenta: Evidence for a human chorionic "growth hormone prolactin". Nature (Lond.) **199**, 1005 (1963).

SOFFER, L.J., SALVANESCHI, J.: A gonadotropin inhibiting substance in the urine of normal subjects. J. clin. Endocr. **22**, 532 (1962).

STEWART, H.L., SANA, M.E., MONTGOMERY, T.L.: Hormone secretion by human placenta grown in tissue culture. J. clin. Endocr. **8**, 175 (1948).

WILDE, C.E., BAGSHAWE, K.D.: In: CIBA Found. Study Grp. 22, S. 46. London: Churchill 1965.

WILSON, R.B., ALBERT, A., RANDALL, L.M.: Quantitative studies on the production, destruction and elimination of chorionic gonadotrophin in normal pregnancy. Amer. J. Obstet. Gynec. **58**, 960 (1949).

Steroid Hormones

BROWN, H.C., SAFFAN, B.D., HOWARD, C.M., PREEDY, J.R.K.: The renal clearance of endogenous estrogens in late pregnancy. J. clin. Invest. **43**, 295 (1964).

— — PREEDY, J.R.K.: Renal handling of estrogens in late pregnancy. J. clin. Invest. **39**, 975 (1960).

CASSMER, O.: Hormone production of the isolated human placenta. Acta endocr. (Kbh.), Suppl. **45** (1959).

DELL'AQUA, S., MANCUSO, S., WIQVIST, N., RUSE, J.L., SALOMON, S., DICZFALUSY, E.: Studies on the aromatisation of neutral steroids in pregnant women. 5. Metabolism of androst-5-ene-3β-,16α-,17β-triol in the intact foetoplacental unit at midpregnancy. Acta endocr. (Kbh.) **55**, 389 (1967).

DICZFALUSY, E.: Endocrine function of the fetoplacental unit. Fed. Proc. **23**, 791 (1964).

— BORELL, U.: Influence of oophorectomy on steroid excretion in early pregnancy. J. clin. Endocr. **21**, 1119 (1961).

— TILLINGER, K.G., WESTMAN, A.: Studies on oestrogen metabolism in new-born boys. 1. Excretion of oestrone, oestradiol-17 and oestriol during the first few days of life. Acta endocr. (Kbh.) **26**, 303 (1957).

DORFMAN, R.I., UNGER, F.: Metabolism of steroid hormones. New York: Academic Press 1965.

GRUMBACH, M.M., KAPLAN, S.L., VILLEE, D.B., VILLEE, C.A., ZANDER, J., SALOMON, S., BIRD, E.E., WILSON, R., WIQVIST, N., RYAN, R.J., DICZFALUSY, E.: Endocrinology of the foeto-placental unit. In: Proc. 2. Int. Congr. Endocrin., S. 691. Amsterdam: Exc. Med. 1965.

HAMMERSTEIN, J., NEVINNY-STICKEL, J.: Verminderte Oestrogenausscheidung in der Schwangerschaft nach subtotaler Adrenalektomie. Acta endocr. (Kbh.) **48**, 375 (1965).

HARKNESS, R.A., LOVE, D.W.: Studies on the estimation of urinary pregnanetriol during pregnancy and childhood. Acta endocr. (Kbh.) **51**, 526 (1966).

HASKINS, A.L., JR.: Assay of circulating progesterone by ultraviolet spectroscopy. Proc. Soc. exp. Biol. (N.Y.) **73**, 439 (1950).

KLOPPER, A.J., MICHIE, E.A.: The excretion of urinary pregnanediol after the administration of progesterone. J. Endocr. **13**, 360 (1956).

— MCNAUGHTON, M.C.: The identification of pregnanediol in liquor amnii, bile and faeces. J. Endocr. **18**, 319 (1959).

LURIC, A.O., VILLEE, C.A., REID, D.E.: The role of the fetus and placenta in maintenance of plasma progesterone. Amer. J. Obstet. Gynec. **96**, 671 (1966).

PEARLMAN, W.H.: (16-^{3}H)progesterone metabolism in advanced pregnancy and in oophorectomized-hysterectomized women. Biochem. J. **67**, 1 (1957).

SCHWERS, J.: Les oestrogènes. Bruxelles: Arscia 1964.

TOUCHSTONE, J.C., GREENE, J.W., MCELROY, R.C., MURAWEC, T.: Blood estriol conjugation during human pregnancy. Biochemistry **2**, 653 (1963).

WYSS, H.: Oestriol- und Pregnandiolausscheidung in der zweiten Schwangerschaftshälfte. Bern: Huber 1970.

ZANDER, J.: Progesterone in human blood and tissues. Nature (Lond.) **174**, 406 (1954).

— Gestagens in human pregnancy. In: LLOYD, C.W., Rec. progress in the endocrinology of reproduction, S. 255. New York: Academic Press 1959.

— Relationship between progesterone production in the human placenta and the fetus. In: Progesterone and the defence mechanism of pregnancy. CIBA Found. Study Grp. 9, S. 32. Boston: Little Brown 1961.

— Die Hormone der Plazenta. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 33. Stuttgart: Thieme 1967.

— KULLANDER, S.: De novo synthesis of progesterone and 4-pregnene-3-one-20-ol in tissue cultures by human

placenta and experimental ovarian tumors of rats. 2. Int. Congr. Horm. Steroids. Amsterdam: Exc. Med. 1966.

Prolactin

APOSTOLAKIS, M.: The extraction of prolactin from human pituitary glands. Acta endocr. (Kbh.) **49**, 1 (1965).

DIXON, H.B.F.: In: PINCUS, G., THIMANN, K.V., ASTWOOD, E.B., The hormones, Bd. 5, S. 1. New York and London: Academic Press 1964.

FORSYTH, I.A.: Prolactin and placental lactogens. In: GRAY, C.H., BACHARACH, A.L., Hormones in blood, 2. ed., Bd. 1, S. 234. London and New York: Academic Press 1967.

MEITES, J.: In: MARTINI, L., GANONG, W.F.: Neuroendocrinology, Bd. 1, S. 669. New York and London: Academic Press 1966.

SAR, M., MEITES, J.: Effects of progesterone, testosterone and cortisol on hypothalamic prolactin-inhibiting factor and pituitary prolactin content. Proc. Soc. exp. Biol. **127**, 426 (1968).

TURKINGTON, R.W.: Human prolactin. Amer. J. Med. **53**, 389 (1972).

(For further references on prolactin see Chap. V.)

Normal Pregnancy

AITKEN, E.H., PREEDY, J.R.K., ETON, B., SHORT, R.V.: Oestrogen and progesterone levels in foetal and maternal plasma at parturition. Lancet II, 1096 (1958).

BAULIEU, E.E., VIGAN, M., DE, BRICAIRE, H., JAYLE, M.F.: Lack of plasma cortisol and urinary aldosterone in a pregnant woman with Addison's disease. J. clin. Endocr. **17**, 1478 (1957).

BERLINER, D.L., JONES, J.E., SALHANICK, H.A.: The isolation of adrenal-like steroids from the human placenta. J. biol. Chem. **223**, 1043 (1956).

BETTENDORF, G., APOSTOLAKIS, M., VOIGT, K.D.: Darstellung von Gonadotropinen aus menschlichen Hypophysen. Acta endocr. (Kbh.) **41**, 1 (1966).

BRODY, S., CARLSTRÖM, G.: Problems associated with the quantitative immunoassay of human chorionic gonadotropin. In: WOLSTENHOLME, G.E.W., KNIGHT, J., Gonadotropins. CIBA Found. Study Grp. 22, S. 70. London: Churchill 1965.

— WIQVIST, N.: Ovarian hormones and uterine growth. Endocrinology **68**, 971 (1961).

BROWN, J.B.: The relationship between urinary oestrogens and oestrogen production in the body. J. Endocr. **16**, 202 (1957).

CASSMER, O.: Hormone production of the isolated human placenta: Studies on the role of the foetus in the endocrine functions of the placenta. Acta endocr. (Kbh.), Suppl. **45**, 32 (1959).

CRETIUS, K.: Veränderungen des mütterlichen Organismus. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 139. Stuttgart: Thieme 1967.

DICZFALUSY, E.: Chorionic gonadotrophin and oestrogens in the human placenta. Acta endocr. (Kbh.), Suppl. **12** (1953).

— Das Verhalten von Choriongonadotropin und Oestrogenen in der menschlichen Plazenta. III. Symp. Dtsch. Ges. Endokrin., S. 147. Berlin-Göttingen-Heidelberg: Springer 1956.

— Endocrine factors in the human fetoplacental unit. Fed. Proc. **23**, 791 (1964).

— LAURITZEN, C.: Oestrogene beim Menschen. Berlin-Göttingen-Heidelberg: Springer 1961.

FREEDBERG, I.M., HAMOLSKY, M.W., FREEDBERG, A.S.: The thyroid gland in pregnancy. New Engl. J. Med. **256**, 505 (1957).

FRIEDBERG, V.: Physiologische Veränderungen des Gesamtorganismus. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 171. Stuttgart: Thieme 1967.

FRIESEN, H.: Purification of a placental factor with immunological and chemical similarity to human growth hormone. Endocrinology **76**, 369 (1965).

GEMZELL, C.A.: Blood levels of 17-hydroxycorticosteroids in normal pregnancy. J. clin. Endocr. **13**, 898 (1953).

GITLIN, D., KUMATO, J., MORALES, C.: Metabolism and maternofetal transfer of human growth hormone in the pregnant woman at term. J. clin. Endocr. **25**, 1599 (1965).

GRUMBACH, M.M., KAPLAN, S.L., ABRAMS, C.L., BELL, J.J., CONTE, F.A.: Plasma free fatty acid response to the administration of chorionic "growth hormone prolactin". J. clin. Endocr. **26**, 478 (1966).

— WERNER, S.C.: Transfer of thyroid hormone across the human placenta at term. J. clin. Endocr. **16**, 1392 (1956).

HENNEN, G., PIERCE, J.G., FREYCHET, P.: Human chorionic thyrotropin: Further characterization and study of its secretion during pregnancy. J. clin. Endocr. **29**, 581 (1969).

HISAW, F.L., ZARROW, M.X.: The physiology of relaxin. Vitamins Hormones **8**, 151 (1950).

JAILER, J.W., KNOWLTON, A.I.: Stimulated adrenocortical activity during pregnancy in an addisonian patient. J. clin. Invest. **29**, 1430 (1950).

JOSIMOVICH, J.B., ATWOOD, B.L.: Human placental lactogen (HPL), a trophoblastic hormone synergizing with chorionic gonadotropin and potentiating the anabolic effects of pituitary growth hormone. Amer. J. Obstet. Gynec. **88**, 867 (1964).

— KNOBEL, E.: Placental transfer of I^{131} insulin in the rhesus monkey. Amer. J. Physiol. **200**, 471 (1961).

JUNG, G.: Proteolytische Enzyme des menschlichen Eierstocks. Geburtsh. u. Frauenheilk. **22**, 960 (1962).

KAPLAN, S.L., GRUMBACH, M.M.: Studies of a human and simian placental hormone with growth hormone-like and prolactin-like activities. J. clin. Endocr. **24**, 80 (1964).

KELLER, P.J.: Die normale und pathologische Schwangerschaft im Spiegel der Choriongonadotropinausscheidung. Gynaecologia (Basel) **163**, 159 (1966).

— GREUB, H., GERBER, C., SCHREINER, W.E.: Die Proteohormone der menschlichen Plazenta. I. Radioimmunologische Bestimmung und Bedeutung des plazentaren Laktogens (HPL). Schweiz. Z. Gynäk. Geburtsh. **1**, 149 (1970).

KNOBIL, E., JOSIMQVICH, J.B.: Placental transfer of thyreotropic hormone, thyroxine, triiodo-thyroxine and insulin in rat. Ann. N.Y. Acad. Sci. **75**, 895 (1959).

LAIDLAW, J.C., COHEN, M., CORNALL, A.G.: Studies on the origin of aldosterone during human pregnancy. J. clin. Endocr. **18**, 222 (1958).

LARON, Z., PERTZELAN, A., MANNHEIMER, S., GOLDMAN, J., GUTTMAN, S.: Lack of placental transfer of human growth hormone. Acta endocr. (Kbh.) **53**, 687 (1966).

LAURITZEN, C.: Das Choriongonadotropin. Gynäk. Rdsch. **3**, 1, 81, 161 (1966).

MASON, N.R., MARSH, I.M., SAVARD, K.: An action of gonadotropin in vivo. J. biol. Chem. **237**, 1801 (1962).

MIGEON, C.J., PRYSTOWSKY, H., GRUMBACH, M.M., BYRON, M.C.: Placental passage of 17-OH-steroids: Comparison of levels in maternal and fetal plasma and effect of ACTH and hydrocortisone administration. J. clin. Invest. **35**, 488 (1956).

MOLEN, H.J., VAN DER: Determination of plasma progesterone during pregnancy. Clin. chim. Acta **8**, 943 (1963).

NEHER, R., STARK, G.: Nachweis von Corticosteroiden in menschlicher Plazenta und Isolierung von 16α-Hydroxytestosteron. Experientia (Basel) **17**, 510 (1961).

PHILIPP, E.: Die Bildungsstätte des Hypophysenvorderlappenhormons in der Gravidität. Zbl. Gynäk. **54**, 450 (1930).

RICE, B.F., HAMMERSTEIN, J., SAVARD, K.: Steroid hormone formation in the human ovary. II. Action of gonado-

tropin in vitro in the corpus luteum. J. clin. Endocr. **24**, 606 (1964).
ROTHMAN, S.: Physiology and biochemistry of the skin. Chicago: Chicago Univ. Press 1954.
RYAN, K.: Hormones of the placenta. Amer. J. Obstet. Gynec. **84**, 1695 (1962).
— Estrogens: Blood and placental levels and the factors, which control them. In: Proc. 2. Int. Congr. Endocrin., S. 727. Amsterdam: Exc. Med. 1965.
SALOMON, S., WATANABE, M., DOMINGUEZ, O.V., GRAY, M.J., MEEKER, C.I., SIMS, E.A.H.: Progesterone and aldosterone secretion rates in pregnancy. Exc. Med. Int. Congr. Ser. **51**, 267 (1962).
SANDBERG, A.A., SLAUNWHITE, W.R.: Transcortin: a corticosteroid binding protein in plasma. II. Levels in various conditions and the effects of estrogens. J. clin. Invest. **38**, 1290 (1959).
SCHOUWEN, M.: Skin and pregnancy. In: TEN BERGE, B.S., Pregnancy. Springfield: Thomas 1964.
SHEARMAN, R.P.: Some aspects of the urinary excretion of pregnanediol in pregnancy. J. Obstet. Gynaec. Brit. Cwlth **66**, 1 (1959).
SHORT, R.V., ETON, B.: Progesterone in blood. III. Progesterone in the peripheral blood of pregnant women. J. Endocr. **18**, 418 (1959).
— LEVETT, I.: The fluorimetric determination of progesterone in human plasma during pregnancy and the menstrual cycle. J. Endocr. **25**, 239 (1962).
STARK, G.: Aldosteron und Schwangerschaft. In: Aldosteron, S. 40. Berlin-Göttingen-Heidelberg: Springer 1963.
TAUBERT, H.D., HASKINS, A.L.: Intravenous infusion of progesterone in human females: blood levels obtained and effect on labor. J. Obstet. Gynec. **22**, 405 (1963).
TOMINAGA, T., TROEN, P.: Stimulation of aromatization in human placenta by human placental lactogen. J. clin. Invest. **46**, 1124 (1967).
TOROK, I.: Die endokrinen Beziehungen der Anencephalie. Acta morph. Acad. Sci. hung. **1**, 237 (1951).
TROEN, P.: Perfusion studies of the human placenta. II. Metabolism of C^{14}-17-B-estradiol with and without added human chorionic gonadotropin. J. clin. Endocr. **21**, 895 (1961).
— MIGUET, M., ALONSO, C.: Sulfurylation of estriol by perfused human placentas: A preliminary report. Biological and clinical aspects of placental steroidogenesis. Int. Congr. Horm. Steroids, S. 63, Milan, 1962.
TURKINGTON, R.W., TOPPER, Y.J.: Stimulation of casein synthesis and histological development of mammary gland by human placental lactogen in vitro. Endocrinology **79**, 175 (1966).
VARANGOT, J., CEDARD, L., YANNOTTI, S.: The biosynthesis of estriol in vitro placental perfusion. Biol. and clinical aspects of placental steroidogenesis. Int. Congr. Horm. Steroids, S. 72, Milan, 1962.
WIDE, L.: An immunological method for the assay of human chorionic gonadotrophin. Acta endocr. (Kbh.), Suppl. **70**, 41 (1962).
WIELE, R.L. VAN DE, GURPIDE, E., KELLY, W.G., LARAGH, S., LIEBERMAN, S.: The secretory rate of progesterone and aldosterone in normal and abnormal late pregnancy. Acta endocr. (Kbh.), Suppl. **51**, 35 (1960).
ZANDER, J.: Die Hormonbildung in der Plazenta und ihre Bedeutung für die Frucht. Arch. Gynäk. **198**, 113 (1962).
— Die bedrohte Schwangerschaft. Die Behandlung der bedrohten Schwangerschaft. Arch. Gynäk. **204**, 92 (1967).
— Die Hormone der Plazenta. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J.: Gynäkologie und Geburtshilfe, Bd. 2, S. 33. Stuttgart: Thieme 1967.
ZARROW, M.X., HOLMSTROM, E.G., SALHANICK, H.A.: The concentration of relaxin in the blood serum and other tissues of women during pregnancy. J. clin. Endocr. **15**, 22 (1958).

Pathological Pregnancy

Abortion

FUCHS, F., STAKEMANN, G.: Die Fehlgeburt. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 1, S. 733. Stuttgart: Thieme 1969.
HERTIG, A.T., ROCK, J.: A series of potentially abortive ova recovered from fertile women prior to the missed menstrual period. Amer. J. Obstet. Gynec. **58**, 968 (1949).
— — ADAMS, E.C.: A description of 34 human ova within the first 17 days of development. Amer. J. Anat. **98**, 435 (1956).
HOFMANN, D.: Die Fehlgeburt. München: Urban & Schwarzenberg 1967.
JOEL, C.A.: Zur Ätiologie des habituellen Abortes unter Berücksichtigung des männlichen Faktors. Gynaecologia (Basel) **154**, 257 (1962).
KÄSER, O.: Zur Ätiologie und Therapie der habituellen Aborte. Schweiz. med. Wschr. **91**, 352 (1961).
KELLER, P.J.: Die normale und pathologische Schwangerschaft im Spiegel der Choriongonadotropinausscheidung. Gynaecologia (Basel) **163**, 159 (1966).
KERR, M., RASHAD, M.N.: Chromosome studies on spontaneous abortions. Amer. J. Obstet. Gynec. **94**, 322 (1966).
KISTNER, R.W.: Gynecology. Chicago: Yearbook med. Publ. 1964.
SHETTLES, L.B.: The ovum in infertility, abortion and development anomaly. Fertil. Steril. **7**, 561 (1956).

Toxemia

BERGER, M., CAVANAGH, D.: Toxemia of pregnancy—The hypertensive effect of acute experimental placental ischemia. Amer. J. Obstet. Gynec. **87**, 293 (1963).
COYLE, M.G., GREIG, M., WALTER, J.: Blood progesterone and urinary pregnanediol and oestrogens in foetal death from severe pre-eclampsia. Lancet **1962II**, 275.
FRIEDBERG, V., HOCHULI, E.: Schwangerschaftstoxikosen. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 455. Stuttgart: Thieme 1967.
KELLER, P.J., GERBER, C., SOLTERMANN, R.: Die Proteohormone der menschlichen Plazenta. III. Die HPL-Aktivität im Schwangerenserum bei Hypertonie, Spätgestose und Praeklampsie. Schweiz. Z. gynäk. Geburtsh. **2**, 109 (1971).
KUMAR, D.: Tissue progesterone concentrations of placentas in pre-eclamptic patients. Amer. J. Obstet. Gynec. **95**, 594 (1966).
KYANK, H., SCHUBERT, E., GYOEMYOESSY, A.: Auswertung der Eklampsie-Sammelstatistik der Jahre 1957–1960 aus 72 deutschen Frauenkliniken. Geburtsh. Frauenheilk. **23**, 961 (1963).
LORAINE, J.A., MATTHEW, G.D.: Chorionic gonadotrophin in toxaemias of pregnancy. J. Obstet. **57**, 542 (1950).
— — The placental concentration of chorionic gonadotrophin in normal and pathological pregnancy. J. Obstet. **60**, 641 (1953).
OGDEN, E., PAGE, W.E.: Rise of blood pressure during ischemia of the gravid uterus. Proc. Soc. exp. Biol. (N.Y.) **43**, 49 (1940).
ROY, E.J., HARKNESS, R.A., KERR, M.G.: Concentration of oestrogens in blood and urine of patients suffering from pre-eclampsia. J. Obstet. Gynaec. Brit. Cwlth **70**, 597 (1963).
SIMMER, H., SIMMER, J.: Progesteron im peripheren Venenblut von Schwangeren mit Spätgestosen. Klin. Wschr. **37**, 971 (1959).
STAEMMLER, H.J.: Untersuchungen über die Regulation des Kortikosteroidhaushaltes in der Schwangerschaft und ihre Störungen. Z. Geburtsh. Gynäk. **145**, 288 (1956).

STARK, G.: Aldosteron und Schwangerschaft. In: Aldosteron. Berlin-Göttingen-Heidelberg: Springer 1963.
VENNING, E.H.: Endocrine aspects of toxemia. In: Clinical obstetrics and gynecology, Bd. 1, Nr 2. New York: Hoeber 1958.

Rhesus Incompatibility, Plural Pregnancy, Prolongation, Fetal Malformations

BENIRSCHKE, K., BLOCH, E., HERTIG, A.T.: Concerning the function of the fetal zone of the human adrenal gland. Endocrinology **58**, 598 (1956).
BORTH, R., LUNENFELD, B., STAMM, O., DE WATTEVILLE, H.: Gonadotrophin and steroid excretion in a case of twin pregnancy. Acta obstet. gynec. scand. **38**, 417 (1959).
KUBLI, F., SPIELMANN, W.: Morbus haemolyticus-Antenatale Aspekte. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 1051. Stuttgart: Thieme 1967.
LANMAN, J.D.: The adrenal gland in the human fetus. Pediatrics **27**, 140 (1961).
MARTIUS, H.: Unser Verhalten bei verlängerter Tragzeit. Dtsch. med. Wschr. **89**, 322 (1964).
MICHIE, E.A.: Urinary oestriol excretion in pregnancies complicated by suspected retarded intrauterine growth, toxaemia or essential hypertension. J. Obstet. Gynaec. Brit. Cwlth **74**, 896 (1967).
ROSENKRANZ, K.D.: Hormonbefunde an Plazenten bei Übertragung. Arch. Gynäk. **168**, 51 (1939).
SCHWERS, J.: Les oestrogènes au cours de la seconde moitié de la grossesse. Bruxelles: Arscia 1965.
SHORT, R.V., ETON, B.: Progesterone in blood. III. Progesterone in the peripheral blood of pregnant women. J. Endocr. **18**, 418 (1959).
WYSS, H.: Oestriol- und Pregnandiolausscheidung in der zweiten Schwangerschaftshälfte. Bern: Huber 1970.

Hydatidiform Mole and Chorionepithelioma

FRANDSEN, A.V., STAKEMANN, G.: The excretion of hormones in cases of hydatiform mole and chorionepithelioma. Acta endocr. (Kbh.), Suppl. **90**, 81 (1964).
HÖRMANN, G.: Zur Systematik einer Pathologie der Placenta. Arch. Gynäk. **191**, 297 (1958).
— LEMTIS, H.: Die menschliche Plazenta. In: SCHWALM, H., DÖDERLEIN, G., Klinik der Frauenheilkunde und Geburtshilfe, Bd. 3. München: Urban & Schwarzenberg 1965.
KAISER, R.: Die diagnostische Verwertbarkeit von Pregnandiolbestimmungen in der Schwangerschaft. Geburtsh. Frauenheilk. **13**, 513 (1953).
KELLER, P.J.: Die normale und pathologische Schwangerschaft im Spiegel der Choriongonadotropinausscheidung. Gynaecologia (Basel) **163**, 159 (1966).
NOVAK, E., SEAH, C.S.: Benign trophoblastic lesions in Methieu chorinepithelioma registry. Amer. J. Obstet. Gynec. **68**, 376 (1954).
SCHOPPER, W., PLIESS, G.: Über Chorionepitheliosis. Ein Beitrag zur Genese, Diagnostik und Bewertung ektopischer chorionepithelialer Wucherungen. Virchows Arch. path. Anat. **317**, 347 (1949).
SCOTT, J.S.: Blasenmole und Chorionepitheliom. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 1, S. 761. Stuttgart: Thieme 1969.

Hormonal Control of Birth

CAROY, H.M.: Myometral activity. In: CAROY, H.M., Modern trends in human reproductive physiology, S. 257. London: Butterworth 1963.
COUTINHO, E.M.: Progesterone and ions. In: MARTINI, L., PERCILE, A., Hormonal steroids, S. 223. New York: Academic Press 1965.
COUTINHO, M.E.: Hormone induced ionic regulation of labor. Proc. 2. Int. Congr. Endocrin. Int. Congr. Series 83, S. 742. Amsterdam: Exc. Med. Found. 1965.
CSAPO, A.I.: Regulation of myometral function. In: WOLSTENHOLME, G.E.W., KNIGHT, J., Progesterone, Its regulatory effect on the myometrium. CIBA Found. Study Grp. 34, S. 20. London: Churchill 1969.
— WIEST, W.G.: An examination of the quantitative relationship between progesterone and the maintenance of pregnancy. Endocrinology **85**, 735 (1969).
JÖCHLE, W., BROWN, W., HIDALGO, M.A., SICKLES, J.: Mechanism of parturition in cattle and swine: Effect of synthetic corticosteroids. Symp. Dtsch. Ges. Endokrin. **16** (1970) (im Druck).
JUNG, H.: Ursache und Zeitpunkt des Geburtseintritts. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 516. Stuttgart: Thieme 1967.
MARSHALL, J.M.: The effects of ovarian hormones on the uterine response to adrenergic nerve stimulation and to adrenergic amines. In: WOLSTENHOLME, G.E.W., KNIGHT, J., Progesterone: Its regulatory effect on the myometrium. CIBA Found. Study Grp. 34, S. 89. London: Churchill 1969.
PORTER, D.G.: Progesterone and the guinea-pig myometrium. In: WOLSTENHOLME, G.E.W., KNIGHT, J., Progesterone: Its regulatory effect on the myometrium. CIBA Found. Study Grp. 34, S. 79. London: Churchill 1969.
PULKKINEN, M.O.: The significance of progesterone in myometrial regulation during mid-trimester human pregnancy. In: WOLSTENHOLME, G.E.W., KNIGHT, J., Progesterone: Its regulatory effect on the myometrium. CIBA Found. Study Grp. 34, S. 133. London: Churchill 1969.
TURNBULL, A.C., ANDERSON, A.B.M.: The influence of the foetus on myometrial contractility. In: WOLSTENHOLME, G.E.W., KNIGHT, J., Progesterone: Its regulatory effect on the myometrium. CIBA Found. Study Grp. 34, S. 106. London: Churchill 1969.
— — WILSON, G.R.: Maternal urinary oestrogen excretion as evidence of a foetal role in determining gestation at labour. Lancet **1967 II**, 627.
WOLSTENHOLME, G.E.W., KNIGHT, J.: Progesterone: Its regulatory effect on the myometrium. CIBA Found. Study Grp. 34. London: Churchill 1969.

Postpartum Period

BROWN, J.B.: Urinary excretion of oestrogens during pregnancy, lactation and the reestablishment of menstruation. Lancet **1956 I**, 704.
BRUN DEL RE, R., DEL POZZO, E., DE GRANDI, P., FRIESEN, H.G., HINSELMANN, M.: Prolactin inhibition and suppression of puerperal lactation by a Br-ergocryptine (CB 154): a comparison with estrogen. Obstet Gynec (in print).
CANFIELD, C.J., BATES, R.W.: Nonpuerperal galactorrhea. New Engl. J. Med. **273**, 897 (1965).
FLÜCKIGER, E., WAGNER, H.R.: 2-Br-α-Ergokryptin: Beeinflussung von Fertilität und Laktation bei der Ratte. Experientia (Basel) **24**, 1130 (1968).
KELLER, P.J.: Excretion of follicle-stimulating and luteinizing hormone during lactation. Acta endocr. (Kbh.) **57**, 529 (1968).
PEAKE, G.T., MCKEEL, D.W., JARETT, L.: Ultrastructure, histologic and hormonal characterization of a prolactin-rich human pituitary tumor. J. clin. Endocr. **29**, 1383 (1969).

RIMOIN, D.L., HOLZMANN, G.B., MERIMEE, T.J.: Lactation in the absence of human growth hormone. J. clin. Endocr. **28**, 1183 (1968).
ROTH, J., GLICK, S.M., CUATRECASAS, P., HOLLANDER, C.S.: Acromegaly and other disorders of growth hormone secretion. Amer. intern. Med. **66**, 760 (1967).
ROY, E.J., HARKNESS, R.A., KERR, M.G.: The concentration of oestrogens in maternal peripheral blood during and after labour. J. Obstet. Gynaec. Brit. Cwlth **70**, 1034 (1963).
SHARMANN, A.: Aspects of the reproductive physiology of the post-partum period. In: BOWES, K., Obstetrics and Gynecology. New York: Hoeber 1956.
SHORT, R.V., ETON, B.: Progesterone in blood. III. Progesterone in the peripheral blood of pregnant women. J. Endocr. **18**, 418 (1959).
SULMAN, F.G.: Hypothalamic control of lactation. Monographs on Endocrin., Vol. 3. Berlin-Heidelberg-New York: Springer 1970.
TALWALKER, P.K., MEITES, J.: Mammary lobulo-alveolar growth induced by anterior pituitary hormones in adreno-ovariectomized and adreno-ovariectomized hypophysectomized rats. Proc. Soc. exp. Biol. (N. Y.) **107**, 880 (1961).
WENNER, R.: Physiologische und pathologische Laktation. Arch. Gynäk. **204**, 171 (1967).
WHITTLESTONE, W.G.: Physiologie of lactation. In: CAREY, H.M., Modern trends in human reproductive physiology, S. 113. London: Butterworth 1963.
ZANDER, J.: Die Hormone der Plazenta. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 33. Stuttgart: Thieme 1969.
ZILLIACUS, H.: Physiologie und Pathologie des Wochenbettes. In: KÄSER, O., FRIEDBERG, V., OBER, K.G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 966. Stuttgart: Thieme 1969.
— SIEMER, H.: Physiologie und Pathologie der Laktation. In: KÄSER, O., FRIEDBERG, V., OBER, K. G., THOMSEN, K., ZANDER, J., Gynäkologie und Geburtshilfe, Bd. 2, S. 998. Stuttgart: Thieme 1969.

Endocrinological Methods of Investigation during Pregnancy

APOSTOLAKIS, M., VOIGT, K.D.: Bioassay of various gonadotrophins. Acta endocr. (Kbh.) **28**, 54 (1958).
— — Gonadotropine. Stuttgart: Thieme 1965.
ASCHHEIM, S., ZONDEK, B.: Die Schwangerschaftsdiagnose aus dem Harn (durch Hormonnachweis). Klin. Wschr. **7**, 8 (1928).
BLOBEL, R.: Über das Choriongonadotropin. Fortschr. Geburtsh. Gynäk., Bd. 28. Basel: Karger 1966.
BRODY, S., CARLSTRÖM, G.: Estimation of human chorionic gonadotrophin in biological fluids by complement fixation. Lancet **1960 II**, 99.
BROWN, J.B., COYLE, M.G.: Urinary excretion of oestriol during pregnancy. I. A shorted procedure. J. Obstet. Gynaec. Brit. Cwlth **70**, 219 (1963).
CRABBEN, H. VAN DER, KAISER, E., WERNER, C., WERNERS, P.H., POTTHOFF, S., ALBRECHT, S., RIESS, H.J.: Hormonelle Belastung der fetoplazentaren Einheit zur Früherkennung der Plazentainsuffizienz. Symp. Dtsch. Ges. Endokrin. **16**, Abstr. 3 (1970).
DICZFALUSY, E.: An improved method for the bioassay of chorionic gonadotrophin. Acta endocr. (Kbh.) **17**, 58 (1954).
DORFMAN, R. I.: Methods in hormone research, 2. ed., Bd. 1. New York and London: Academic Press 1968.
FRIEDMAN, M.H., LAPHAM, M.E.: Simple, rapid procedure for laboratory diagnosis of early pregnancies. Amer. J. Obstet. Gynec. **21**, 405 (1931).
GALLI-MAININ, C.: Pregnancy test with male batrachia. Endocrinology **43**, 349 (1948).
ITTRICH, G.: Eine Methode für die klinische Routinebestimmung der Harnoestrogene. Zbl. Gynäk. **82**, 429 (1960).
JACOBS, L.S., MARIZ, I.K., DAUGHADAY, W.H.: A mixed heterologous radioimmunoassay for human prolactin. J. clin. Endocr. **34**, 484 (1972).
JAYLE, M.F., SCHALLER, R., JARRIGO, P., MITAY, S.: Hydrolyse des phénolstéroides conjugués urinaires. Bull. Soc. Chim. biol. (Paris) **41**, 1593 (1959).
KELLER, P.J., BAERTSCHI, U., BADER, P., GERBER, C., SCHMID, S., SOLTERMANN, R., KOPPER, E.: Biochemical detection of fetoplacental distress in risk prognancies. Lancet II, 729 (1971).
— SCHREINER, W.E.: Erste Erfahrungen mit der DHEA-S-Belastung zur foetoplazentaren Funktionsdiagnostik. Schweiz. Z. Geburtsh. Gynäk. **1**, 331 (1970).
KLOPPER, A.J.: Pregnanediol and pregnanetriol. In: DORFMAN, R.I., Methods in hormone research, 2. ed., Bd. 1, S. 229. New York and London: Academic Press 1968.
— MICHIE, E.A., BROWN, J.B.: A method for the determination of urinary pregnanediol. J. Endocr. **12**, 209 (1955).
KOVACIC, N.: The deciduoma assay. A method for measuring prolactin. J. Endocr. **28**, 45 (1963).
KUPPERMANN, H.S., GREENBLATT, R.B., NOBACK, C.R.: Two and six-hour pregnancy test; preliminary report. J. clin. Endocr. **3**, 548 (1943).
KWA, H.G., VERHOFSTAD, F.: Radioimmunoassay of rat prolactin. Biochim. biophys. Acta (Amst.) **133**, 186 (1967).
LAURITZEN, C.: Conversion of DHEA-sulfate to estrogens as a test of placental function. Horm. Met. Res. **1**, 96 (1969).
LORAINE, J.A.: The estimation of chorionic gonadotrophin in the urine of pregnant women. J. Endocr. **6**, 319 (1950).
— Human chorionic gonadotropin. In: GRAY, C.H., BACHARACH, A.L., Hormones in blood, 2. ed., Bd. 1, S. 313. London and New York: Academic Press 1967.
OERTEL, G.W.: Chemische Bestimmung von Steroiden im menschlichen Harn. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
PARLOW, A.F.: A rapid bioassay method for LH and factors stimulating LH secretion. Fed. Proc. **17**, 402 (1958).
PAULSEN, C.A.: Estrogen assays in clinical medicine. Seattle: University of Washington Press 1965.
PREEDY, J.R.: Estrogens. In: DORFMAN, R.I., Methods in hormone research, Bd. 1, S. 1. New York and London: Academic Press 1968.
SAXENA, B.N., REFETOFF, S., EMERSON, K., SELENKOW, H.A.: A rapid radioimmunoassay for human placental lactogen. Amer. J. Obstet. Gynec. **101**, 874 (1968).
WIDE, L., GEMZELL, C.A.: An immunological pregnancy test. Acta endocr. (Kbh.) **35**, 261 (1960).

XII. Disorders of Sexual Differentiation (Intersexuality)

A. PRADER

A. Definition and Terminology

Strictly speaking, *intersexuality* signifies the presence of intersexual external genitalia, i.e. genitalia which are neither definitely male nor definitely female. In the broader sense, however, all disorders of prenatal sexual differentiation belong to intersexuality. The almost incalculable multiplicity of abnormal sexual differentiation can be divided into three major groups:

1. *Abnormal gonadal development.* This is commonly the result of an abnormal set of chromosomes which in turn frequently leads to abnormal genital development.

2. *Abnormal genital development in the presence of normal testes* and usually normal male set of chromosomes (male pseudohermaphroditism).

3. *Abnormal genital development in the presence of normal ovaries* and normal female set of chromosomes (female pseudohermaphroditism).

The traditional classification of abnormal sexual differentiation into true hermaphroditism, male pseudohermaphroditism and female pseudohermaphroditism does not include the entire spectrum of this classification. *True hermaphroditism* with ovarian and testicular tissue is only one of the many forms of abnormal gonadal development. In contrast, the term *male pseudohermaphroditism* correctly includes abnormal genital development in the presence of normal testes (male intersexuality, androgynia), and the term *female pseudohermaphroditism* includes abnormal genital development in the presence of normal ovaries (female intersexuality, gynandria).

Finally, the acquired forms of intersexuality, in which heterosexual symptoms arise for the first time postnatally, can also be classified under intersexuality, in the broader sense of the word. Hirsutism and virilization are present in the female sex, and gynaecomastia and feminization in the male sex. These acquired syndromes are not dealt with here but in the Chapters on the adrenals, testes and ovaries.

B. Embryology

The manner and stages of normal sexual differentiation known today can be summarized in a simple scheme (Fig. 1). The sex chromosomes (XX in the woman, and XY in the man) decide whether male or female gonadal differentiation occurs. The gonads in turn regulate prenatal genital differentiation, postnatal development of the secondary sexual characteristics and also psychosexual development to a certain degree. Animal experiments in recent years have shown that testosterone from fetal or neonatal testes exerts an irreversible branding effect on the brain (HARRIS, 1965).

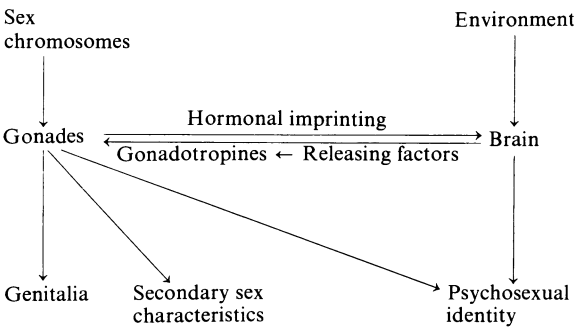


Fig. 1. Sex differentiation

In animal experiments, this branding or lack of it determines the future sexual behavior, settles the question of cyclic or non-cyclic female gonadotropin production in the future and thus influences fertility. It is not yet known whether this basically important process of branding is also applicable to man. The psychosexual development in man is a very complex process which is not only determined by anatomical and hormonal factors but by effective psychological environmental influences as well.

Gonadal differentiation (Fig. 2) occurs during the first few embryonal months. Undifferentiated gonads consist of cortex and medulla. In the boy, the medulla differentiates into the testis between the 6th and 8th weeks. This differentiation

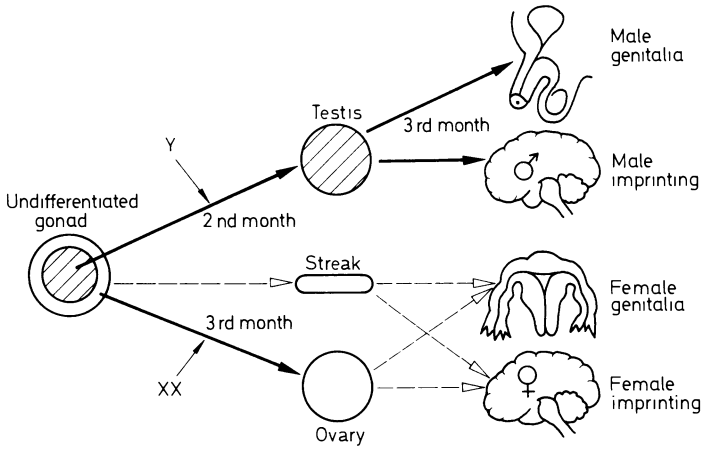


Fig. 2. Gonadal and genital differentiation

necessitates the presence of a Y chromosome. Differentiation of the medulla into the ovary occurs a few weeks later provided two X chromosomes are present and Y chromosomes absent. Normal gonadal differentiation is presumably also dependent on the presence of primordial germ cells. It is not known whether antagonistic inductors in the cortex and medulla play a part during normal gonadal differentiation, or whether the presence of a single inductor alone is sufficient to cause testicular differentiation, and absence of this inductor results in ovarian differentiation. In man, there are no known hormonal disorders of gonadal differentiation whereas in certain bovine races an intersexual form termed a "freemartin" is known, which may possibly be due to hormonal factors. The chromosomal male twin of heterosexual twin calves develops normally, whereas the chromosomal female twin is subjected to intersexual gonadal and genital development and is later similar to an animal subjected to early castration in stature, sexual instinct and fertility. Prenatal circulatory anastomoses presumably have some part in the development of this intersexual form. Despite intensive research the exact cause has still not been explained.

Genital differentiation in the male sex is regulated by biochemical factors of the fetal testes, whereas similar influences are not present in the fetal ovary in the female (Fig. 2). The famous animal experiments by JOST and clinical experience have shown that genital differentiation is also of the female type when fetal testes are completely absent or are present as undifferentiated streaks (p. 714). In summary, male genital development is an active, induced process and female genital development an autonomous passive process.

Genital differentiation is schematically presented in Fig. 3, and the homologous genital structures in man and woman are given in Table 1. Genital differentiation reaches a neutral stage at the end of the second fetal month. The internal genital system consists of Wolffian ducts and Müller's ducts, and the external genitalia of the urogenital sinus and genital eminences. In the boy, the ductus deferens, epididymis and seminal vesicle develop from the Wolffian duct during the 3rd month, while Müller's duct degenerates. In the girl, the Wolffian duct

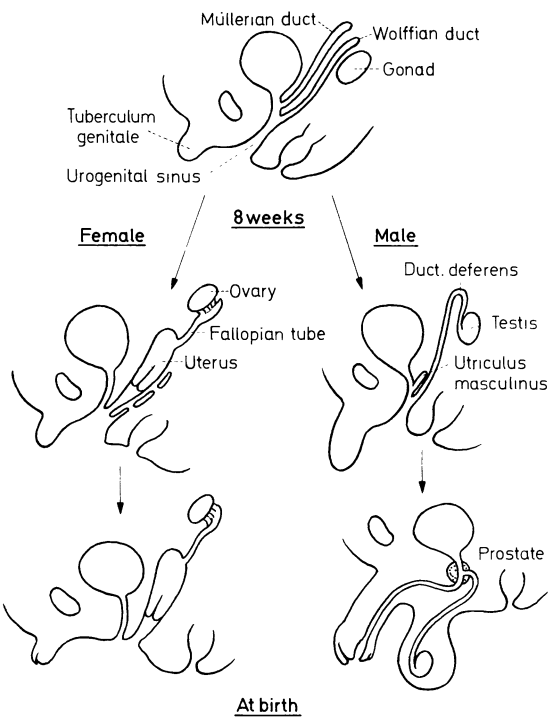


Fig. 3. Genital differentiation

Table 1. Homologous genital structures in man and woman

Male	Undifferentiated stage	Female
Testis Seminiferous tubules Rete testis	Gonads Cortex Medulla	Ovary Cortex Rete ovarii
Epididymis Efferent ductules Appendix epididymis	Epigenitalis (upper group of Mesonephric ducts)	Epoopheron Appendix vesiculosa
Paradidymis Aberrant ductule	Paragenitalis (lower group of mesonephric ducts)	Paroopheron
Ductus epididymidis Ductus deferens Glandula vesiculosa Ductus ejaculatorius	Wolffian duct	Gartner's duct
Appendix testis Utriculus masculinus	Müller's duct	Tuba uterina Uterus Vagina
Colliculus seminalis	Müller's cumulus	Hymen
Urethra (distal of coll. seminalis) Prostate Bulbo-urethral glands	Urogenital sinus	Vestibulum Para-urethral glands Vestibular glands (BARTHOLINI)
Penis	Genital protuberances	Clitoris
Scrotum Scrotal raphe	Genital tori	Labia majora Posterior commissure
Corpus cavernosum urethrae	Genital folds	Labia minora

disappears, whereas the uterus, tube and upper vagina arise from Müller's duct. Development of the external genitalia in the boy is characterized by growth of the genital eminence into the penis, fusion of the genital folds to form the penile urethra and fusion of the genital tori to form the scrotum. In the girl, the labia minora arise from the genital folds and the labia majora from the genital tori.

Active induction of male genital differentiation is presented in Fig. 4. Our knowledge is based largely on experimental work done by JOST in animals, but clinical experience shows that it is applicable to the human as well. The fetal testis acts in three ways on genital development, it stimulates the differentiation of the Wolffian duct to a vas deferens and suppresses the differentiation of Müller's duct into the tube and the uterus.

Induction of external male genital development is due to the action of androgenic hormones. This effect can be imitated by testosterone and is probably exerted by testosterone produced by Leydig cells in the fetus. The vasa deferentia develop from the Wolffian ducts, and in the presence of only one testis, only one vas deferens develops, on the same side as the testis and never on the contralateral side. This effect can be simulated by direct contact with a testosterone crystal but not by an injection of testosterone. Obviously, a local active factor is involved, which presumably is the high

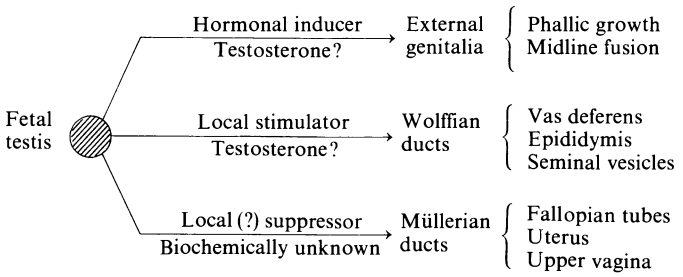


Fig. 4. Differentiation of male genitalia (9th to 13th week)

concentration of testosterone in the Leydig cells of the fetal testis. Suppression of tubal and uterine development from Müller's ducts cannot be imitated by means of testosterone. Here, a still unknown biochemical factor of the fetal testis is involved, this factor being independent of testosterone production.

Thus the fetal testis exerts an androgenic effect; in low concentrations the androgenic hormones stimulate external genital development, and in high concentrations they stimulate development of the vas deferens. On the other hand, the fetal testis has another effect, independent of its other actions, which suppresses the development of the tube and uterus. Absence of androgenic hormones (p. 726) and failure to suppress Müller's ducts (p. 725) are known in human pathology. Such disorders result in the development of male pseudohermaphroditism. There are also abnormal androgenic influences (p. 730) known to affect female genital development and resulting in female pseudohermaphroditism.

C. Methods of Investigation

Investigation should clarify the genital state, chromosomes, gonadal relations and the pathogenesis of the disorder. Investigation of the internal genital organs is difficult, chromosome investigations time-consuming and direct examination of the gonads only possible by means of a laparotomy or laparoscopy. For these reasons, an attempt is first made to solve the problem by indirect methods by relating genetic and anamnestic data, and by a general physical examination and estimation of the sex chromatin. Costly chromosome investigations and exploratory laparotomies are necessary only in selected cases.

The *history* often offers valuable help. Inquiries must be made about similar cases in the family history, about primary amenorrhoea in the case of women, and a special attempt must be made to determine whether there has been any history of an adrenogenital syndrome in siblings (vomiting during babyhood, acceleration of somatic development, virilization). In the personal history, it is important to find out whether genital peculiarities were obvious at the time of birth, how somatic development proceeded during the first years of life, at what time and to what degree secondary sexual characteristics arose, how long the female patient has been menstruating and whether the patient has already undergone genital and intra-abdominal operations and herniotomy. If possible, one should try to obtain a picture of the intellectual, psychic and psychosexual development.

General physical examination must in particular determine exact body measurements, special characteristics of stature, dysmorphic signs, malformations and the degree of development of secondary sexual characteristics.

An exact *psychopathological examination*, with special reference to psychosexuality, is essential after the second year of life.

External genital findings are of extreme importance. There are merging transitional stages between pure female and pure male external genital development, and we have arbitrarily divided them into five stages (Fig. 5). Type I (Fig. 5) shows enlargement of the clitoris without other genital changes. In Type

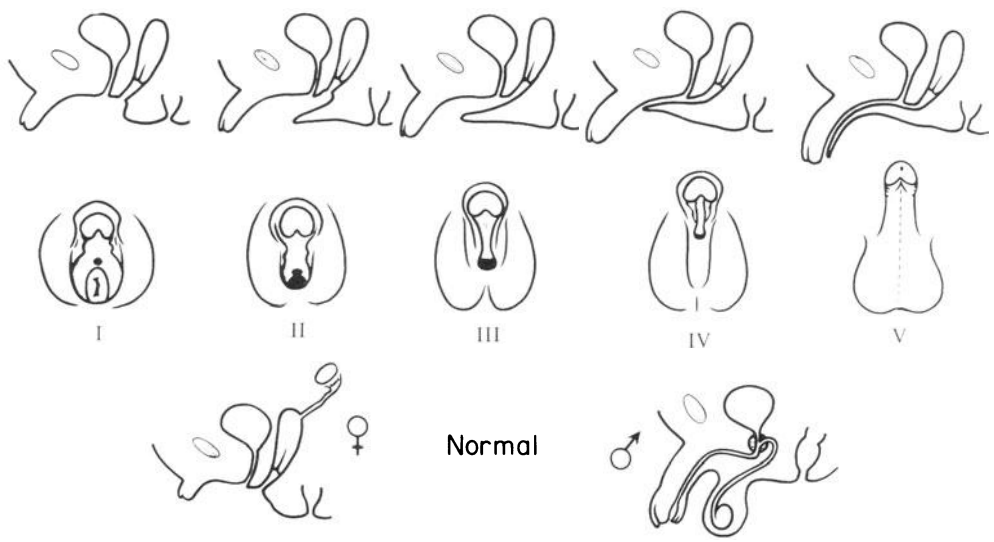


Fig. 5. Intersexual external genitalia, Types I–V

II (Fig. 5), in addition to clitoric enlargement, there is a wide funnel-shaped urogenital sinus which contains the openings of the vagina and urethra. In Type III (Fig. 5), the urogenital sinus is narrower but still funnel-shaped. In Type IV (Fig. 5), only a small urogenital opening can be seen at the base of the phallus. In Type V (Fig. 5), the urogenital opening is found at the tip of the phallus as in the normal male. Perineal hypospadias corresponds to Type IV, whereas hypospadias of the glans and penis lies somewhere between Type IV and V. The various types can be explained by androgenic stimulation varying qualitatively and quantitatively. Outward appearances do not indicate whether the sex hormones and gonads are male, female or abnormal, and no diagnosis can be made about the original disorder. The view that the aspect of external genitalia does not permit diagnosis of the cause is of fundamental importance. In boys, Types I–IV represent inadequate androgenic stimulation. In girls, Types III–V are only possible if abnormal androgenic stimulation is present as early as the 3rd month. Quite mild androgenic stimulation, or perhaps androgenic stimulation arising for the first time after birth, can result in Type 1.

Investigation of the internal genital state is more difficult. If there is only one urogenital opening, then the question arises of whether a vagina opens in the urogenital sinus lying posteriorly, or whether a male urethra with a typical colliculus seminalis is present. As a rule, this question can be answered by means of a sound, ureteroscopy and X-rays using contrast media (urethro-vaginogram). If there are separate urethral and vaginal openings, the depth of the vagina can be determined by introducing a sound. Direct vaginal examination, with a urethroscope in the child and with a speculum in the adult, and rectal palpation will show if a uterus is present or not.

Estimations of the excretion of 17-ketosteroids and when possible of testosterone in the urine and plasma are the most important *hormonal* investigations for recognizing androgenic influence. When an adrenogenital syndrome is suspected, estimation of pregnanetriol excretion usually gives some diagnostic indication (p. 369). The question of estrogenic influences arises only during puberty and can be assessed just as well by means of the secondary sexual characteristics and the vaginal smear (p. 535) as with the aid of measurements of urinary estrogens. Estimation of gonadotropins is of great value. After the appearance of the first secondary sexual characteristics, biological assay of urinary gonadotropins permits differentiation between high levels in primary gonadal insufficiency (hypergonadotropic hypogonadism, p. 460) and lowered values in hypophyseal insufficiency (hypogonadotropic hypogonadism, p. 469). This investigation is pointless at an earlier stage since the method is not sensitive enough. With new radioimmunological methods for measuring FSH and LH in urine and blood, it is often possible to recognize abnormally high or abnormally low values even before puberty.

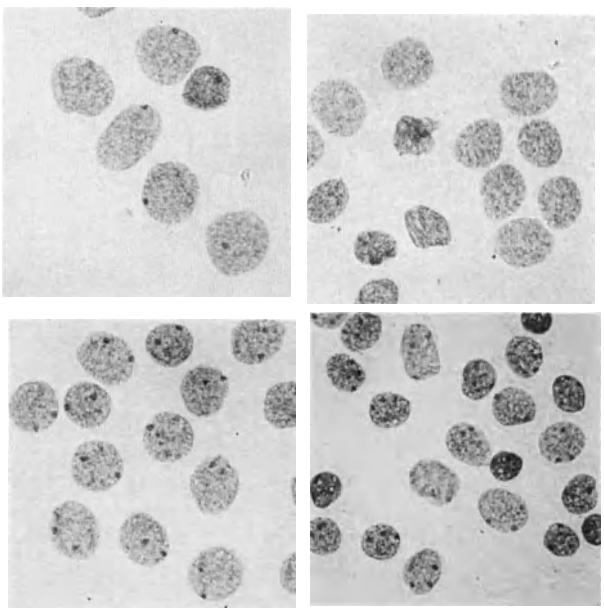


Fig. 6. Sex chromatin in the hair root: Upper left; chromatin-positive in normal XX woman; upper right; chromatin-negative in normal XY man; lower left; double sex chromatin in XXX girl; lower right; triple sex chromatin in XXXY boy. (From SCHMID, 1967)

Investigation of the sex chromatin in the buccal smear or hair root (Fig. 6) yields information about the number of X chromosomes present. This investigation is based on the fact that only one X chromosome is genetically active whereas additional X chromosomes are genetically inactive and only genetically inactive X chromosomes are visible as sex chromatin bodies. The number of X chromosomes thus exceeds the maximal number of sex chromatin bodies per cell nucleus by one. Sex chromatin findings reveal nothing about the number of Y chromosomes. Thus, a limited number of karyotypic possibilities are still open which can be determined through direct chromosomal examination. Recently however, *fluorescent staining of the Y chromosome* with quinacrinedihydrochloride (Atebrin) has been recommended (PEARSON, 1970). This investigation is just as

simple as examining sex chromatin, and the number of Y chromosomes present can be recognized directly. The two investigations are complementary and it should be possible in the future to assess the set of sex chromosomes without the costly direct chromosome examination.

In the presence of an abnormal constellation of sex chromosomes, it is possible to determine in certain cases, such as color blindness and Xg blood groups of patients and members of the family, from the X chromosomal properties how this chromosomal disorder arose and whether it was due to a chromosomal disturbance during oogenesis in the mother or during spermatogenesis in the father (RACE and SANGER, 1969).

Investigation of *dermatoglyphics* can also be of interest where a disorder of the sex chromosomes is suspected. The number of skin grooves of the finger pulp is inversely proportional to the number of sex chromosomes, i.e. increased in the presence of XO, lowered with XXY and greatly reduced with XXXXY etc. (PENROSE, 1968).

Direct determination of the gonadal sex is only possible by gonadal biopsy or the detection of spermatozoa in the ejaculate. Usually however, the diagnosis can be closely limited by consideration of the other findings so that there is no longer any doubt about the gonadal sex and the gonadal biopsy becomes superfluous. This is true for most of the classic syndromes such as Klinefelter's syndrome, Turner's syndrome, testicular feminization, adrenogenital syndrome etc. Biopsy of the gonads is thus only seldom necessary.

D. Review and Classification

There is presently no truly satisfactory classification for the numerous disorders of prenatal sexual differentiation, since etiology and pathology of the various forms are only partly clarified. The classification used below is based partly on anatomical and partly on pathological aspects. Abnormal gonadal development is discussed on p. 712, male pseudohermaphroditism on p. 724 and female pseudohermaphroditism on p. 730. The individual syndromes are also indicated in the same sections.

E. Abnormal Gonadal Development

The synopsis in Table 2 gives a survey of the numerous forms of abnormal gonadal differentiation. Hypergonadotropic primary hypogonadism is present in all forms (p. 460) and with few exceptions is associated with sterility resistent to therapeutic measures.

As has been mentioned, these disorders are frequently but not always the result of a sex chromosome anomaly. Like chromosome disturbances, most of these syndromes are therefore not hereditary. However, the reverse conclusion must not be drawn that every disorder of the sex chromosomes is associated with abnormal gonadal development and sterility. Normal gonadal development and normal fertility are usually found in two common syndromes, the XXX and XYY syndromes.

The chromatin-positive, true Klinefelter's syndrome (p. 464) is the most common syndrome among *patients with male external genitalia.* In the majority of cases, an XXY karyotype is the cause. A similar clinical picture with a normal XY karyotype is termed *chromatin-negative, pseudo-Klinefelter's syndrome* (p. 468). The pathogenesis of the syndrome XX men is the least understood of the various syndromes

Table 2. Disorders of prenatal sexual differentiation

A. Abnormal gonadal development

Disorders of sex chromosomes common.
Majority of syndromes not hereditary.

1. Male external genitalia

- Chromatin-positive Klinefelter's syndrome: usually XXY.
- Chromatin-negative Klinefelter's syndrome: XY. Very heterogeneous group.
- XX men.
- Male Turner's syndrome: usually XY. Heterogeneous group.
- Congenital anorchia: XY.

2. Female external genitalia

- Turner's syndrome (gonadal dysgenesis) with bilateral streaks and dwarfism: usually XO, rarely XXqi, XO/XX and many others, occasionally transition to rudimentary ovaries or rudimentary testes and to asymmetrical gonadal dysgenesis.
- Pure gonadal dysgenesis with bilateral streaks without dwarfism: XX form with autosomal recessive mode of inheritance and XY form inherited though healthy women, occasionally transition to rudimentary ovaries or rudimentary testes.

3. Intersexual external genitalia

- Asymmetric mixed gondal dysgenesis with one streak and one rudimentary testis: usually XO/XY, less frequently XY or XX/XY, occasionally transition to Turner's syndrome, pure XY-gonadal dysgenesis and to rudimentary testes (dysgenetic form of male pseudohermaphroditism).
- True hermaphroditism. Fully differentiated ovarian and testicular tissue, usually XX, less commonly XX/XY etc.
- Agonadism. No gonads and no internal genital structures:
  XY. Hereditary?

Table 2 (continued)

B. Abnormal genital development in presence of normal testes = Male pseudohermaphroditism

Usually normal male XY karyotype.

Most syndromes hereditary.

1. Male external genitalia

- Oviduct persistence (hernia uteri inguinalis): autosomal recessive heredity.

2. Female external genitalia

- Testicular feminization ("hairless women"): Heredity through healthy women.
- Disorders of testosterone synthesis (see below).

3. Intersexual external genitalia

Very large group, little explained. Numerous cases before puberty unclassifiable.

- Disorders of testosterone synthesis: autosomal recessive heredity of enzyme defect.
  - In testes and adrenals: 20-hydroxylase, 3β-dehydrogenase, 17-hydroxylase.
  - In testes alone: 17-reductase, 17-desmolase.
- Incomplete testicular feminization with female pubertal development: heredity via healthy women.
- Hereditary vulviform perineal hypospadias: autosomal recessive heredity.
- Chromosome disorders XO/XY.
- Dysmorphic syndrome: Smith-Lemli-Opitz syndrome, Russel's dwarfism, Ullrich-Feichtinger syndrome, trisomia 13 etc.

C. Abnormal genital development in the presence of normal ovaries = female pseudohermaphroditism

Normal female XX karyotype.

Male or intersexual external genitalia

- Congenital adrenogenital syndrome: various autosomal recessive heredities of enzyme defects: 3β-dehydrogenase, 11β-hydroxylase, 21-hydroxylase.
- Transplacental virilization through exogenous steroids: administration of androgens or synthetic gestagens during pregnancy.
- Transplacental virilization due to androgenic tumors in the mother: ovarian and adrenocortical tumors.
- Complex urogenital malformations, renal agenesis.
- Idiopathic hypertrophy of the clitoris.

of this group. Anorchia (p. 461) and the syndrome of prepubertal castration (p. 461), i.e. the absence of testes in otherwise normal boys, must be due to secondary destruction of the testes after the 3rd fetal month, since otherwise induction of the external genitalia would not have been male (p. 708).

Turner's syndrome is most common in *patients with female external genitalia* (p. 713). It is characterized by the combination of rudimentary dysgenetic gonads or "streaks" with dwarfism and various dysmorphic features. The classical karyotype is XO, although many other karyotypes can occur. In *pure gonadal dysgenesis* (p. 721), which is a rare condition, there is an XY or XX karyotype present, but anatomical and functional gonadal findings are exactly the same, whereas dwarfism and dysmorphic characteristics found in TURNER's syndrome are missing.

Abnormal gonadal development in *patients with intersexual external genitalia* is seen particularly with *mixed or asymmetric gonadal dysgenesis* (p. 721) and in *true hermaphroditism* (p. 722).

1. Turner's Syndrome (Gonadal Dysgenesis)

This syndrome was first described by MORGAGNI as far back as in 1760, and involves individuals with internal and external normal female genitalia and functionless rudimentary gonads (streaks). In addition to the two obligatory findings, *dwarfism* and *hypogonadism*, a dysmorphic syndrome is often associated with a sphinx face, pterygium formation at the neck, shield-shaped thorax and other characteristics. Dwarfism, the dysmorphic syndrome and hypogonadism together produce a characteristic clinical picture. The dysmorphic syndrome by itself was previously also known as the Bonnevie-Ullrich condition, but this term was stretched so far clinically that it led to more confusion than clarity. Together with hypogonadism, this syndrome was later termed as ovarian agenesis or Turner's syndrome (TURNER, 1938) or Ullrich-Turner syndrome. Since rudimentary gonads are present and show neither specific ovarian nor specific testicular elements, the term ovarian agenesis is not suitable. Today, the terms Turner's syndrome and gonadal dysgenesis are usually used, although the term gonadal dysgenesis is also used for the syndrome of asymmetric mixed gonadal dysgenesis, and by some authors even for other syndromes, such as for example Klinefelter's syndrome.

a) Genetics, Chromosomal Findings and Pathogenesis

An X chromosome is missing in the majority of cases. The *karyotype* is therefore 45,XO. The *sex chromatin* is correspondingly *negative*. Investigations of the Xg blood group in female patients and parents have shown that the X chromosome present is usually derived from the father (RACE and SANGER, 1961), i.e. an ovum with a normal X content is fertilized by a sperm without a sex chromosome or one X chromosome is lost again after fertilization. In agreement with this, it is found that the mean maternal age is not raised, contrary to the situation in certain other chromosome

disorders (Down's syndrome, Klinefelter's syndrome).

In addition to the classic XO karyotype, there are other karyotypes with structural disturbances or mosaics. About a third of all cases of TURNER's syndrome belong to this group. Patients in this group are chromatin-positive almost without exception. The most common structural disorders are the iso-chromosome (XXqi), the absence of the short arm (XXp-) and the ring chromosome (XXr). The XO/XX karyotype is the most frequent form of mosaic. There are other more complicated forms of mosaics but usually XO is a part of them. An intact Y chromosome is never found in a classic Turner's syndrome but a structurally abnormal Y (XYpi or XYqi) is found in rare cases. Further, a clinical syndrome similar to Turner's syndrome is occasionally seen in association with an XO/XY mosaic (p. 721).

The *development of individual symptoms* has only been partly explained. The absence of two whole X chromosomes is the cause of the gonadal dysgenesis and explains the female genital development which occurs without exception (p. 708) and the hypogonadism. The dysmorphic features and dwarfism are not results of the hypogonadism, or they could not be present even before puberty. Everything indicates that they are a direct result of the absence of the short arm of an X chromosome (or long arm of the Y chromosome) (FERGUSON-SMITH, 1961). This presumably also applies to the osteoporotic-like bone dystrophia which can be determined before puberty as well.

As is to be expected with chromosome disorders, *affection of siblings is extremely rare.* In two cases, an XO female patient had a normal XY twin brother (TURPIN, 1961). In another case, an XO/XX mosaic was observed in mother and daughter (DE MEYER, 1970).

b) Incidence

The incidence of the chromatin-negative Turner's syndrome among newly born girls is 1 : 2700 (TAYLOR, 1967). Since a third of all the cases of Turner's syndrome found later on are chromatin-positive, the total incidence is about 1 : 1800. The incidence is much higher in spontaneous abortions, there being one chromatin-negative case in 18 abortions (PAWLOWITZKI, 1966). This signifies that the XO Turner's syndrome is very common in embryos and usually acts as a prenatal lethal factor.

c) Pathology

The gonads are inconspicuous cord-like or strip-like structures of a whitish colour, lying in the normal ovarian position (Fig. 7). In abortuses with the XO karyotype, normal ovarian differentiation is seen as with the XX karyotype, in the third month with primordial ova and pregranulosa cells. Later, these elements disappear, leaving behind connective tissue similar to the ovarian stroma, containing a few rudimentary mesonephric tubules and quite frequently also nests of epitheloid cells (paraganglionic cells, hilar cells, interstitial cells). These cell nests occur in both chromatin-negative and chromatin-positive patients. Graafian follicles and seminiferous tubules are completely absent in classic cases. The incidence of ovarian tumours is not increased. Internal genital organs are normally formed but the uterus and tubes remain hypoplastic even in adulthood. The adrenals are as a rule normal.

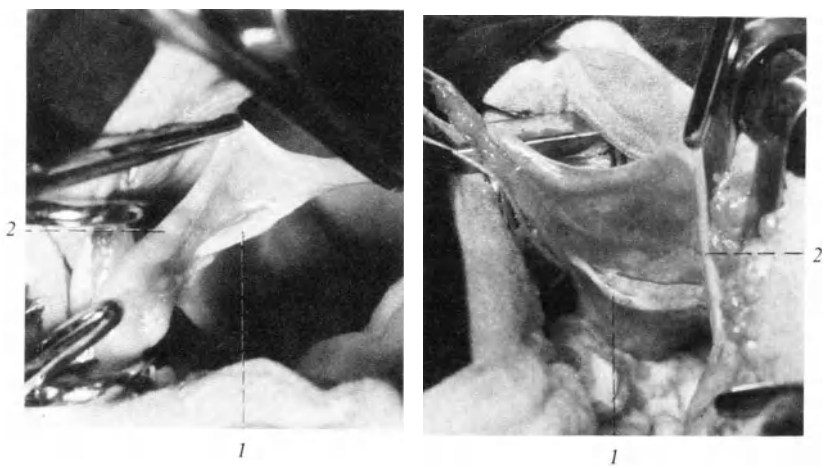
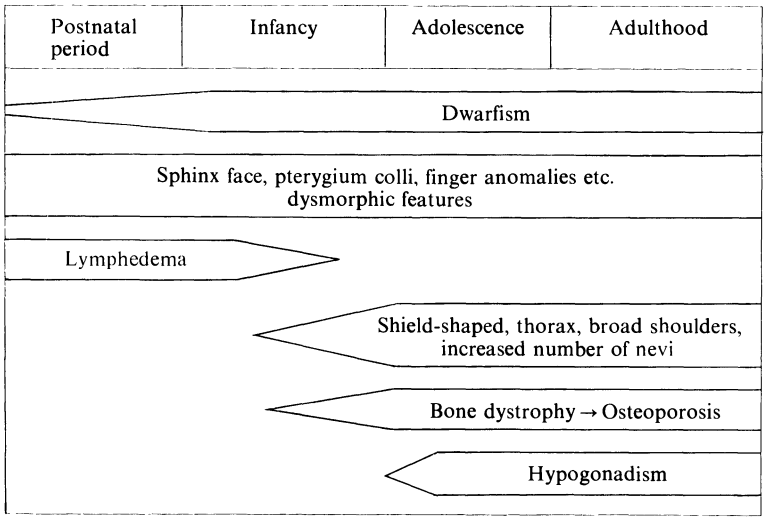


Fig. 7. Streak gonads in Turner's syndrome. **1** gonads; **2** Fallopian tube. (From HAUSER, 1961)

Table 3. Symptoms of TURNER's syndrome dependent on age



d) Clinical Features

Symptoms depend on the age of the patients and on the intensity of dysmorphic characteristics (Table 3). Since these features are often more conspicuous and are noticed much earlier than hypogonadism, they are described first in the fully developed stage, although they are only facultative features in contrast to hypogonadism which is obligatory.

Dwarfism is occasionally already present at the time of birth. More frequently however, these children are of normal size at birth but then simply grow more slowly so that the retardation in growth becomes increasingly obvious. In addition, there is no pubertal spurt in growth due to the hypogonadism. The adult patient is usually 130–150 cm tall. During childhood, the bone age is only insignificantly behind the child's age. In young adults, however, epiphyseal fusion and thus cessation of growth are frequently delayed by 3–4 years, but the growth deficit is not compensated for during this delay.

Face and neck show a series of typical features giving rise to a sphinx-like picture (Figs. 8 and 9): anti-mongoloid slant of the eyes, epicanthus, short nose, large, low-set, poorly shaped ears, carp-like mouth, narrow high palate, short jaw (micrognathia), broad short neck, pulling pterygium colli from insertion of ear to shoulder, and low broad hair line on the neck. Ptosis, pareses of ocular muscles, minor malformations of the eyes and glaucoma are quite common findings. Chronic otitis is also common.

The shoulders are often conspicuously broad. The thorax in the older child and adult, and frequently also even at an earlier age, is obviously short and wide and has a flat depression on the anterior aspect, so that one speaks of a shield-shaped thorax (Figs. 10 and 11). The circumference of the thorax is too large compared to the height of the patient. The nipples are often hypoplastic, i.e. even smaller than a baby's, and are strikingly wide apart (Fig. 10).

The *extremities* also show typical features. The elbows are in a slight or accentuated valgus position. Exostotic-like thickening of the medial tibial condyl is often found in the region of the knee. Lymphedema of the dorsum of the hands and feet is not uncommonly found in the baby and infant (Fig. 8). This edema disappears over a few years and is only found exceptionally in the older child. Shortening of the metacarpals and metatarsals is occasionally seen (brady-metacarpia) as in pseudo-hypoparathyroidism. The dorsal flexion folds of the fingers and toes are often strikingly deeply furrowed, and the nails are often small and deeply embedded. Minor finger anomalies, such as partial syndactylia, klinodactylia etc., are also common.

Apart from pterygium colli, the *skin* also shows several peculiarities. In the baby, the skin of the neck is conspicuously flaccid and loose (cutis laxa) (Fig. 8) similar to that seen in mongoloid idiots (Down's syndrome). This feature disappears after babyhood. A large number of small and large *nevi* are seen on the whole body during infancy and adulthood (Fig. 12).

X-rays show thick cord-like bone dystrophy and a single X-ray of the hand alone often suggests the diagnosis. The vertebral column often shows signs of kyphosis with Scheuer-

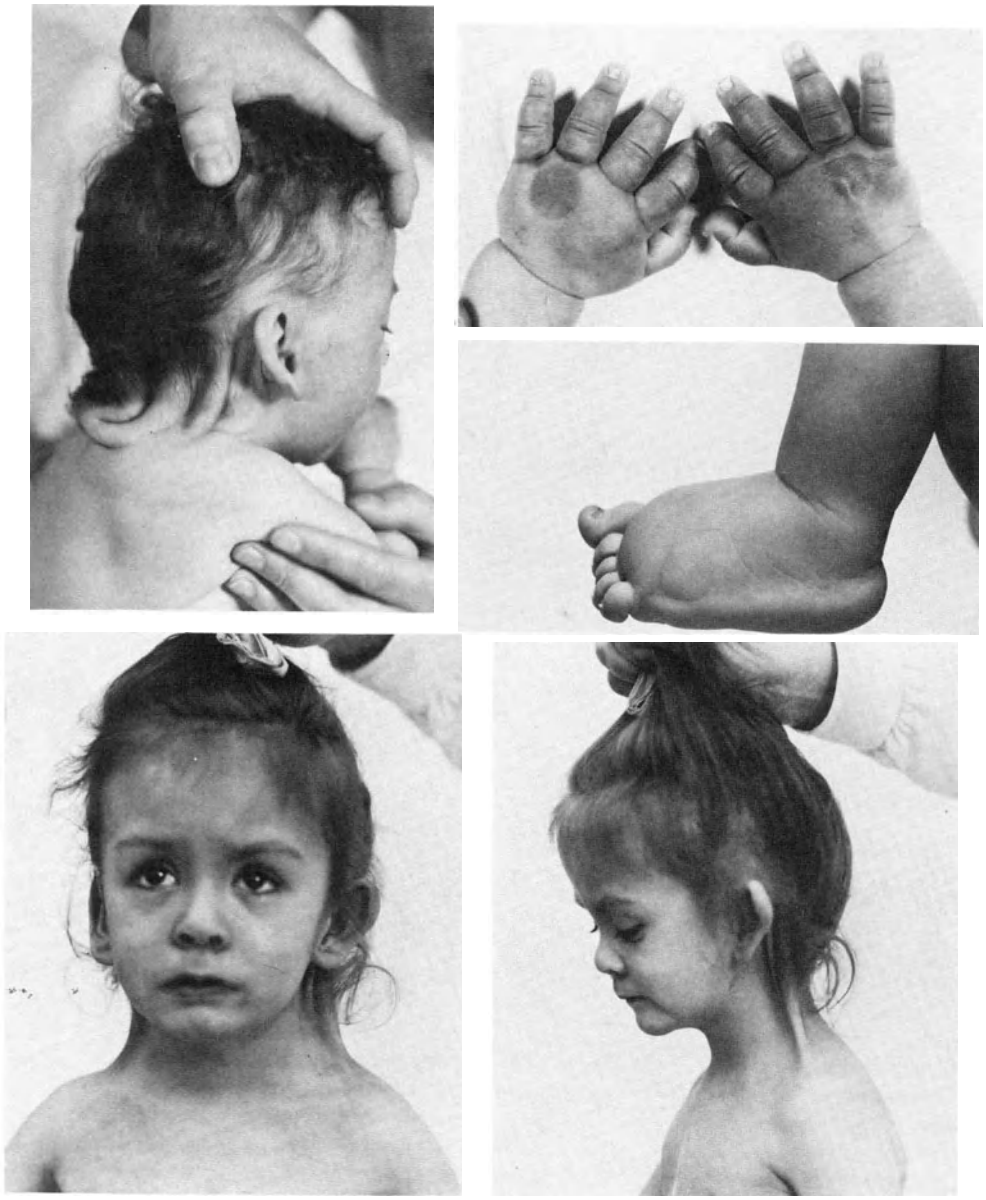


Fig. 8. Girl with XO-Turner's syndrome at ages of 5 months and 2 years. In the baby, loose neck creases, low-set ears, lymphedema of hands and feet and small, deeply embedded nails are typical. At 2 years of age, typical sphinx face with ears inserted low and define pterygium colli. (From PRADER, 1962)

mann-like changes at a very early age, which later become even worse due to true osteoporosis.

Malformations of the kidneys and heart are quite common. Unexplained hypertension in the absence of a cardiac defect or renal malformation is also seen not uncommonly. Anomalies which normally occur less frequently in females than in males are of special interest. *Stenosis of the aortal isthmus* and *red-green color blindness* (an X chromosomal recessive heredity) belong to this group of anomalies, and are both found remarkably frequently in Turner's syndrome.

In contrast to the dysmorphic features described so far, which can occur in various combinations and intensities, *hypogonadism* is obligatory for the diagnosis. It of course only becomes obvious after the 13th or 14th year of life when the expected development of the breasts fails to occur and menarche never takes place. The genitalia remain infantile (Fig. 13), and no estrogenic effects can be found in the vaginal smear. In contrast to the

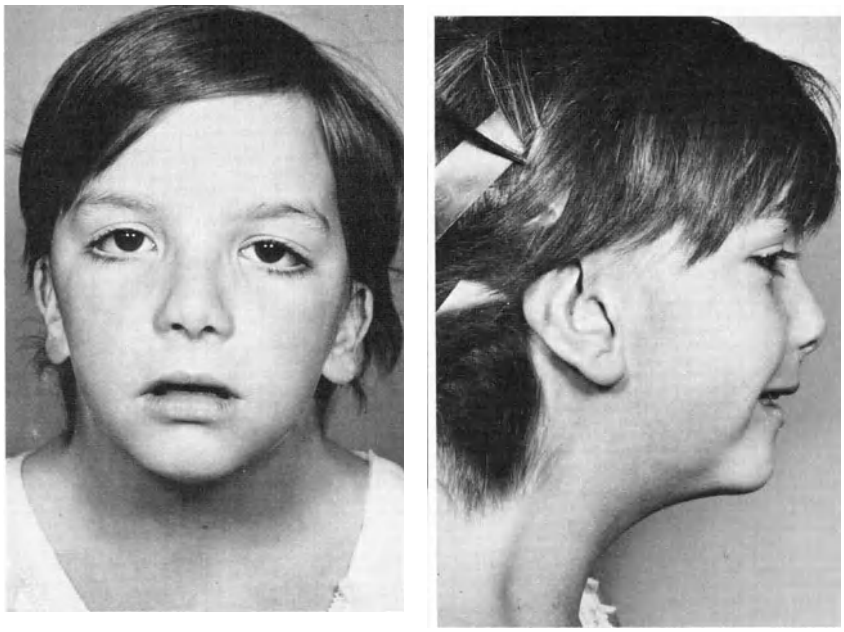


Fig. 9. Typical facial features in Turner's syndrome: antimongoloid slant of the eyes, short nose, large, badly formed ears, short lower jaw, thick, short neck and pterygium colli

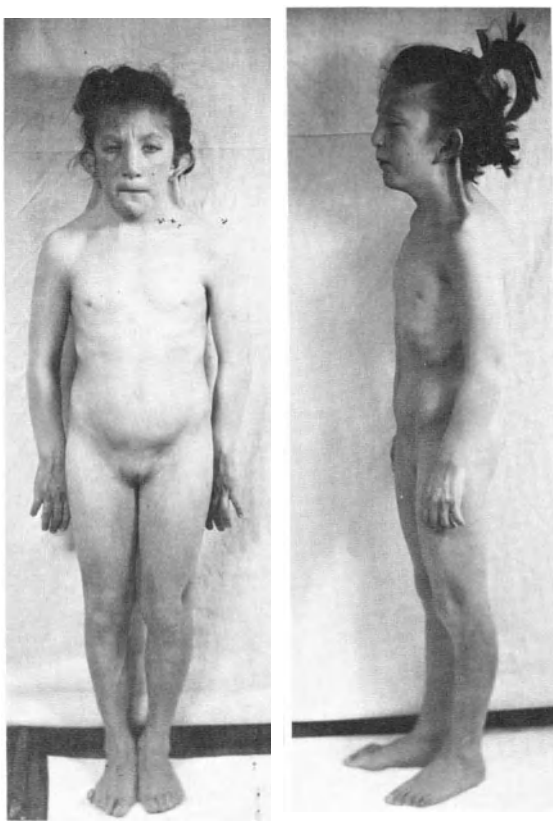


Fig. 10. 12-year-old girl with extreme dwarfism (116 cm) and dysmorphic characteristics (elderly sphinx face, pterygium) of Turner's syndrome

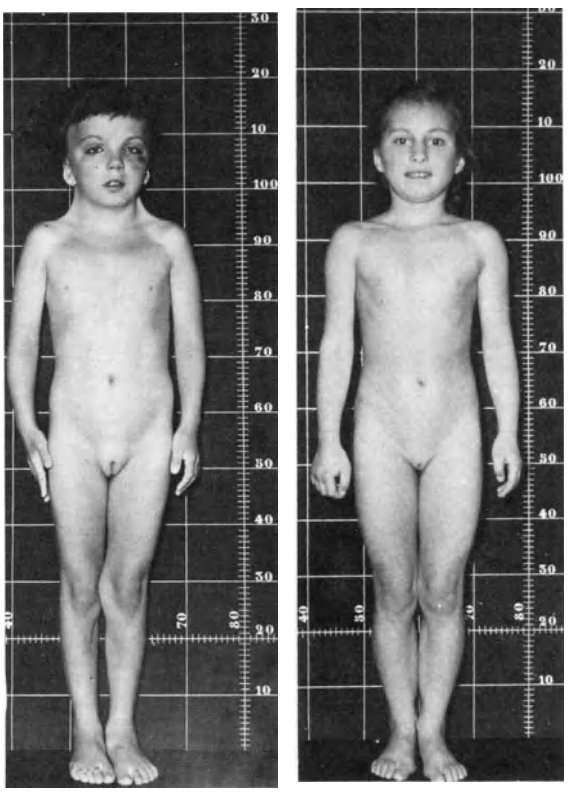


Fig. 11. Two girls of small stature with XO-Turner's syndrome at the ages of 9 and 11 years. Marked dwarfism in both. Dysmorphic characteristics very definite in one (left) and absent in the other (right)

complete absence of estrogenic features, pubic and axillary hair are not entirely absent, but appear later than normal and are poorly developed. It is assumed that this is induced by adrenocortical androgens (Chap. XIX).

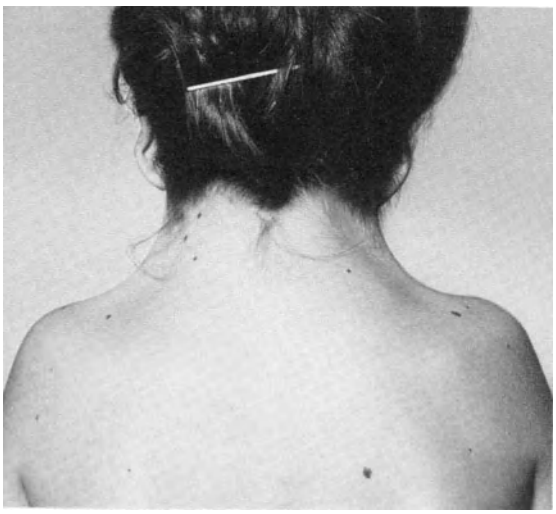


Fig. 12. Neck of a 9-year-old girl with XO-Turner's syndrome. The wide neck, low hair line, pterygium colli and numerous naevi are typical

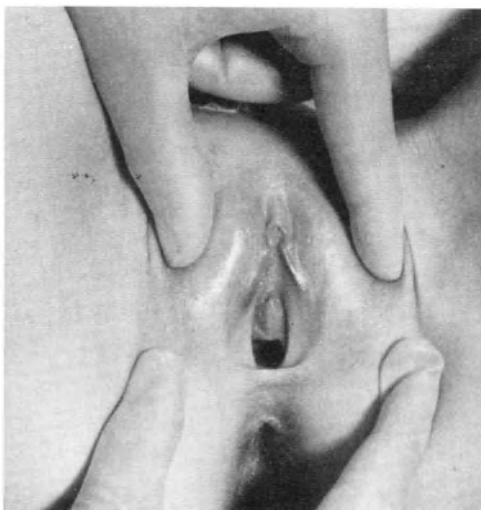


Fig. 13. Infantile genitalia in a 19-year-old female patient with XO Turner's syndrome. (From HAUSER, 1961)

Due to the hypogonadism, fusion of the epiphyses is somewhat delayed in the adolescent and young adult, so that duration of growth is also prolonged. This often results in mild eunuchoid proportions in the adult with body height exceeding arm span, in spite of dwarfism. A further result of estrogen deficiency is osteoporosis which is often found in adults, and which has adverse effects on any changes in the vertebral column, giving rise to back pains.

An increased incidence of diabetes and lymphocytic thyroiditis has been observed repeatedly in adult patients. There is also an increased incidence of thyroid antibodies, which gave rise to some discussion on whether Turner's syndrome or the XO constellation is associated with a predisposition for forming auto-antibodies.

Intelligence is usually somewhat reduced, especially compared with that of other members of the family. The mean intelligence quotient is 85 (ZUEBLIN, 1969). Usually, however, the patients appear more intelligent since they are capable of adjusting well to social conditions. The *psyche* is usually of female nature but shows definite infantile tendencies. The personality is not very striking and there is a lack of initiative and drive. It is interesting to note that stereoscopic perception and orientation are considerably impaired.

The *expectancy of life* is not impaired. Even MORGAGNI's patient reached an age of 66.

As has been mentioned many times already, the clinical picture varies very widely in Turner's syndrome. There is only a very slight interrelation with chromosomal findings which is hardly of any diagnostic value. Nevertheless, the following rough relations between karyotype and the clinical picture can be adhered to: The classic picture is found particularly with karyotype XO. Dwarfism is obligatory. Dysmorphic features are found in most of the XO patients, are usually well developed and seldom completely absent. Hypogonadism is also obligatory, but a few cases can be seen where there is mild development of the breasts and irregular menstruation. In such cases, the gonads are not true streaks but rather hypoplastic ovaries. Fully developed secondary sexual characteristics and fertility have so far only been found in one pure XO case (BAHNER, 1960). Slight hypertrophy of the clitoris is seen in a few cases, as well as marked pubic hair which is probably a result of increased androgen production due to the larger number of hilus cells in the ovaries.

The clinical picture resulting from structural changes in the X chromosome is similar to that seen with the XO karyotype. The situation is different in mosaic cases with normal XX line. Here, dwarfism and hypogonadism can be absent, so that *all transitions between the classic Turner's syndrome and normal female development can occur.*

If one also includes the cases of XO/XY mosaic with rudimentary testes (p. 721) and a few clinical symptoms of Turner's syndrome,

then here as well *all transitions between Turner's syndrome and normal male development can arise.*

e) Hormone Findings

As can be expected from the clinical picture, *estrogens* are greatly reduced in older girls and adult patients. *Gonadotropins* are usually greatly elevated. This finding can be demonstrated even during childhood in the majority of cases, if modern radioimmunological methods of estimation are used and results are compared with normal values for the corresponding ages. Excretion of *17-ketosteroids* and *androgens* is reduced or lies in the lower normal limits. Excretion of dehydroepiandrosterone appears to be somewhat elevated and androsterone excretion somewhat diminished.

f) Diagnosis and Differential Diagnosis

The diagnosis is easy in female patients over 15 years presenting with the fully developed syndrome. The dwarfism, typical external appearance, the primary amenorrhea and manifest hypogonadism immediately suggest this syndrome. If the buccal smear is chromatin-negative, then the diagnosis of an XO Turner's syndrome is confirmed. If the buccal smear is chromatin-positive, examination of the chromosomes is required in order to confirm the diagnosis. Exploratory laparotomies and gonadal biopsies were performed earlier but are no longer justified in classical cases.

The diagnosis is more difficult in *younger patients* since the hypogonadism is not yet recognizable. Diagnostic difficulties can also arise when dysmorphic features are only discretely developed or completely absent. Differential diagnosis of dwarfism, dysmorphic characteristics and hypogonadism are dealt with separately in the next section.

Dwarfism in a girl should always bring the possibility of Turner's syndrome to mind. Discrete characteristics of dysmorphia should then be looked for and the sex chromatin examined in every case to detect at least the more common chromatin-negative cases. Dwarfism combined with hypogonadism also suggests pituitary *dwarfism*. In hypophyseal hypogonadism, dysmorphic features are absent, bone age is usually more retarded and there are usually other signs of hypophyseal insufficiency. In Turner's syndrome, gonadotropin levels are elevated and growth hormone in the plasma can be stimulated by normal measures. In pituitary dwarfism, gonadotropins are normal or diminished and growth hormone levels cannot be stimulated.

In addition to *hypogonadism* in Turner's syndrome, *pure gonadal dysgenesis* and *isolated gonadotropin deficiency* (idiopathic eunuchoidism in the woman) must also be considered. In both cases, there are no signs of dwarfism and dysmorphic features found in Turner's syndrome.

Dysmorphic features when classically developed are extremely characteristic of Turner's syndrome, so that an experienced physician suspects the diagnosis even in the baby and small infant. Often however, these features are so discrete that they must be searched for. Even when the features are well developed, an inexperienced individual can mistake the syndrome for other dysmorphic syndromes and pseudo-Turner's syndrome in particular (see below). In the presence of a Turner-like dysmorphic syndrome with additional dysmorphic features, Turner's syndrome is less probable.

The *pseudo-Turner's syndrome or Noonan's syndrome* is not a very well defined syndrome. Turner-like dysmorphic features are present, but chromosomal disturbances and also hypogonadism as a rule are missing. It occurs in both sexes. The *male Turner's syndrome* can basically also be counted to this group (see p. 720). The syndrome is occasionally familial. The patients are usually but not always of small stature. They show signs of a Turner-like dysmorphic syndrome, but these features usually deviate from Turner's syndrome in single characteristics. In the majority of cases, there is some cardiac malformation. This fact contrasts with findings in Turner's syndrome, but stenosis of the aortal isthmus is never found. In other cases, in addition to the classical lateral pterygium colli, pterygium may also develop in the anterior region of the neck, in the axilla, at the elbow and back of the knee joint. Such cases of the *pterygium syndrome* do not belong to Turner's syndrome. In *arthrogryposis*, dwarfism and dysmorphic features similar to those in Turner's syndrome are found in addition to the stiffening of joints. The sex chromosomes and gonadal function are normal in all these dysmorphic syndromes.

g) Treatment

Whereas sterility cannot be corrected, suitable substitution therapy with female sexual hormones can cause the appearance of secondary sexual characteristics and menstruation at any time. In a few cases, it is questionable whether such treatment really has any sense or whether the patient would not be happier and come through life with less trouble if left untreated.

The internal and external situation must be examined individually for each case before any decision is made.

Substitution therapy should not be commenced before the normal age for the onset of puberty, i.e. not before the 13th–14th year. Since prolonged treatment over decades is involved, oral hormone preparations are preferable. Cyclic treatment with estrogens is the simplest method: 0.5–1.5 mg diethylstilbestrol or 0.05–0.1 mg ethinylestradiol daily for three weeks, followed by a week's break before beginning again. Combined substitution with estrogens and gestagens (p. 601) is better. One of the sequence preparations (p. 568f.) usually used as ovulation inhibitors is today very often used instead.

With this treatment mammary glands, labia minora, vagina and uterus develop as in normal female puberty. However, not uncommonly development of the breast is not quite ideal; the nipples can often become strongly pigmented, and an almost black and crust-like thickening can arise in the mamillary epidermis due to treatment with stilbestrol. Menstruation occurs regularly every 4 weeks. Sexual hair, previously poorly developed, becomes more marked. The cause of this is presumed to be the effect of estrogens leading to increased androgen production by the adrenal cortex, via the pituitary. However, body hair as a whole also increases, although not excessively. Urinary 17-ketosteroids rise to normal values for an adult woman and elevated levels of gonadotropins fall.

If epiphyseal fusion is not yet complete, this form of treatment leads to a definite, but usually modest *acceleration of growth*, but it is not certain if the final height is materially improved. The treatment can at the same time inhibit and favourably influence an osteoporosis already present. Certain authors think that as long as epiphyseal fusion has not yet occurred, growth can be more favourably influenced by administering additional doses of anabolic steroids, or by giving these steroids alone. Unfortunately there is no form of treatment from which definite improvement in growth and the ultimate adult height can be expected.

The infantile psychological characteristics and poor sexuality improve considerably with treatment. The patients become more mature mentally, and steadily realize more or less normal sexuality so that marriage can be successful even in the sexual respect. There is therefore no reason to object to the marriage of a treated patient providing both partners are fully informed about the sterility of the woman.

2. Male Turner's Syndrome

Occasionally, male individuals are seen who have normal male genitalia, but become conspicuous by poor growth and dysmorphic features similar to those found in Turner's syndrome (Fig. 14). This condition is called male Turner's syndrome if hypogonadism is present, and Noonan's syndrome in the absence of hypogonadism. These two states probably belong to a heterogeneous group. The presenting features are the dysmorphic characteristics with pterygium colli, sphinx face and cubitus valgus. A cardiac defect may also occasionally be present. Intelligence is frequently slightly impaired. Puberty is not very marked and the testes remain small because of deficient tubular development. However, absolutely normal testicular development and normal puberty are also often found in the same phenotype.

Examination of the chromosomes usually reveals an XY karyotype. There can be a familial occurrence. An XO/XY mosaic is also found

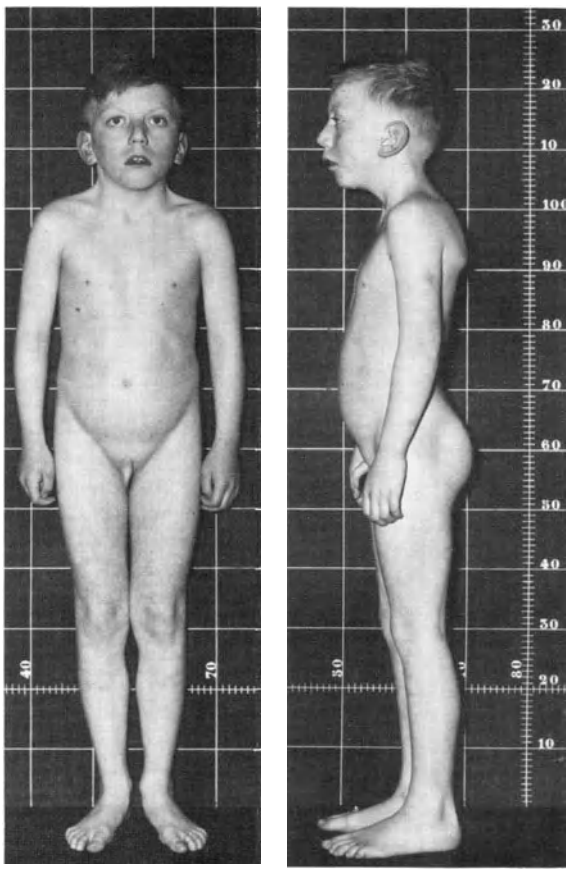


Fig. 14. 11-year-old boy with the male Turner's syndrome: small stature, sphinx face, short and broad neck, small nipples, bilateral cryptorchidism and mild mental deficiency

occasionally. Mixed gonadal dysgenesis with intersexual external genitalia is classically associated with this mosaic. In extreme cases with normal male genital formation and obvious dysmorphic features of Turner's syndrome, it is a matter of opinion whether one uses the term "male Turner's syndrome" or "mixed gonadal dysgenesis".

3. Pure Gonadal Dysgenesis

Pure gonadal dysgenesis, occasionally also termed as Swyer's syndrome, involves individuals with internal and external normal female genitalia and functionless rudimentary gonads (streaks), as does Turner's syndrome. In contrast to Turner's syndrome, neither dwarfism nor dysmorphia is present.

The *karyotype* is XX or XY. There is a familial incidence for both types. The chromatin-positive XX form is probably inherited through an autosomal recessive gene. The chromatin-negative XY form appears to be transmitted only through healthy women so that recessive inheritance of one X chromosome appears probable. In any case, two different genotypes are involved. It is unknown whether sporadic cases belong exclusively to these two genotypes or whether other causal factors are present. In a few cases, the alleged Y may really be an Xq, i.e. an X with deletion of the long arm (KARL, 1967). Absence of male genital differentiation in the XY form can be explained by the absence of fetal testes which are essential for the induction of male genital development (p. 708).

The *gonads* are streaks, similar in appearance to those in Turner's syndrome (p. 714). Histologically, they consist largely of only fibrous stroma. A few follicular elements or accumulations of hilus cells are occasionally found. In the XY form, rudimentary tubules can also be seen occasionally.

Malignant gonadal tumours, gonadoblastomas or dysgerminomas, were found in 7 of 26 XY cases (BARR, 1967).

The striking *clinical feature* is the absence of secondary sexual characteristics or the very poor development of these. Body proportions are often eunuchoid. Enlargement of the clitoris is occasionally found in the XY form. Estrogen excretion is reduced, whereas gonadotropins in the blood and urine are raised.

Asymmetric mixed gonadal dysgenesis must be considered in the *differential diagnosis*. Pure gonadal dysgenesis with an XY karyotype as well as an asymmetric mixed gonadal dysgenesis with an XY or XO/XY karyotype can be present in a chromatin-negative patient of normal height, with hypertrophy of the clitoris and rudimentary testes. In the latter type of dysgenesis, a testis can be found on one side, giving rise to male pubertal development. The danger of tumors is similar in both syndromes.

4. Asymmetric Mixed Gonadal Dysgenesis

This syndrome is applied to individuals with intersexual genitalia, with one testis on one side and a rudimentary gonad (streak) on the other side. Dwarfism and dysmorphic features similar to those in Turner's syndrome may also be observed. There are about 50 known cases so far.

The sex chromosome mosaic XO/XY is the cause of this syndrome in the majority of cases. The XO line explains the rudimentary gonads and the clinical Turner's elements, whereas the XY line explains testicular development and the at least partial male genital development. The XO/XY mosaic probably arises from a Y loss during one of the first mitoses after fertilization. Most cases with the XO/XY mosaic show asymmetric mixed gonadal dysgenesis. In a few cases however, streak gonads are also found bilaterally, associated with a more or less definite Turner's syndrome, or bilateral testes with intersexual genital development, i.e. with the clinical picture of male pseudohermaphroditism are found (p. 724).

In addition to the XO/XY mosaic, there are also cases of asymmetric mixed gonadal dysgenesis with the XY karyotype, XX/XY mosaic and other complicated mosaic forms.

The intersexual external genitalia are the striking *clinical* feature. All stages between mild clitoral hypertrophy and mild hypospadias occur (Fig. 15). Pure female or pure male

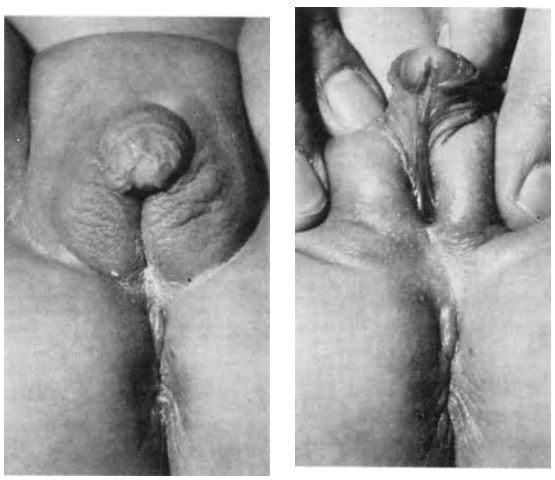


Fig. 15. Intersexual genitalia (Type III) in asymmetric mixed gonadal dysgenesis with XO/XY karyotype

external genitalia are extremely rare. The remaining physical development is not obtrusive or as in Turner's syndrome, dwarfism and Turner-like dysmorphic features such as sphinx face, pterygium colli and shield thorax can be found, though to a lesser degree. Secondary sexual characteristics are usually of the male type but often not very marked. There may be slight development of the breasts but menstruation fails to occur.

The unilateral *testis* usually lies intra-abdominally, but can also occasionally be situated in the inguinal region or scrotum. The degree of differentiation varies, and the testis can be rudimentary or even almost normal. On the other side of the body, a rudimentary streak-type gonad lies in the position of an ovary, as in Turner's syndrome. Occasionally no gonad at all can be found on this side. A rudimentary uterus is usually present. A tube is usually found on the same side as the rudimentary gonad, and a tube or a ductus deferens or both can be found on the side with the testis. The deficient male development of the external genitalia and inadequate suppression of the Müller's derivatives indicate the insufficiency of the fetal testis. Development of *malignant tumors* is common on the side with the streak and the side with the testis. In one study a gonadoblastoma or seminoma was found in 11 of 40 cases (AARSKOG, 1970).

The *diagnosis* of an asymmetric mixed gonadal dysgenesis must therefore always be considered when external genitalia are intersexual, the sex chromatin is negative and when only one gonad or none at all can be palpated in the scrotum or inguinal region. In this situation, surgical exploration with gonadal biopsy is indicated. If asymmetric mixed gonadal dysgenesis is found, the intra-abdominal testis as well as the streak-like gonad should be removed because of the danger of tumors. If the external genitalia are more masculine the child is brought up as a boy and the state of the genitalia corrected surgically. If the genitalia are more of a feminine nature, the child is declared a girl, external genitalia are corrected by appropriate surgical measures, any testicular tissue present is removed, and substitution therapy with female sex hormones is introduced at the age of puberty.

5. XX Men

About 40 individuals have been described with confirmed testes and a pure XX sex chromosomal configuration (DE LA CHAPELLE, 1964, 1970). They show normal male sexual differentiation, normal male stature, normal growth, normal adult height, normal body proportions and normal intelligence. The latter findings contrast to the classic Klinefelter's syndrome. The testes are too small but not always as small as in Klinefelter's syndrome. The condition resembles that in Klinefelter's syndrome in that the secondary sexual characteristics are often not very well developed and gynecomastia is commonly present. Androgen production is correspondingly somewhat reduced and gonadotropin excretion is raised. The histological picture of the testis is sometimes similar to that found in Klinefelter's syndrome and sometimes similar to the picture in aplasia of the germinal cells.

Since according to present day knowledge a testis can only develop in the presence of a Y chromosome, it is difficult to explain how this syndrome develops. An XX/XY mosaic and an XX/XXY mosaic have been discussed, where the line contained in Y has disappeared or is not found on examination. Another theory is that partial exchange occuring between X and Y chromosomes during meiosis could cause the X chromosome to acquire similar testicular-determined properties (X^y) to the Y chromosome (FERGUSON-SMITH, 1966). Since according to LYON'S hypothesis, it is a matter of coincidence whether the X or X^y is active more frequently, either ovaries (normal woman) or testes (XX man) or ovaries and testes (true hermaphroditism) could develop.

6. True Hermaphroditism

The term true hermaphroditism is used when the gonads definitely contain both testicular and ovarian tissue. Over 200 cases have so far been described.

Very little is known about the *etiology and pathogenesis*. Most cases are chromatin-positive. More than half the cases show a normal set of female XX chromosomes. In such cases, the same speculations (partial exchange between X and Y with development of an X^y) can also be adapted for the syndrome with XX men. Bilateral ovotestes have been observed in siblings of two families. The karyotype was measured only in one family and was XX (ROSENBERG, 1963). The XY karyotype and other mosaic forms arise as well as the XX karyotype. The XX/XY mosaic is the commonest mosaic form. In a few cases, two different populations of erythrocytes and plasma proteins have been demonstrated, so that it can be assumed that an ovum with two nuclei is fertilized by two different sperms (dispermia). Of special interest is the observation of a true XX/XY hermaphrodite and an XX man in the same generation

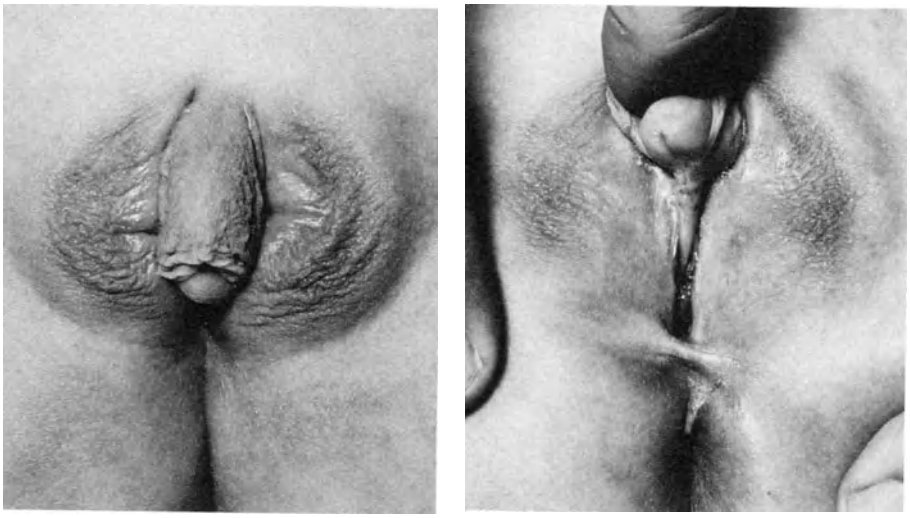


Fig. 16. Intersexual genitalia (Type II) in true hermaphroditism with a testis on the right side and an ovary on the left. (From PRADER, SIEBENMANN, and BETTEX)

of siblings (BERGER, 1970). True hermaphroditism is not always found with an XX/XY karyotype; asymmetric mixed gonadal dysgenesis (see p. 721) or more or less normal testicular development with or without hypospadias (male pseudohermaphroditism, p. 724) can also be associated with this mosaic (AARSKOG, 1970).

The *external genitalia* almost always have an intersexual appearance, Types III and IV (Figs. 5 and 16, p. 710, 723) occur most frequently, so that the majority of patients are brought up as boys. Purely male or purely female external genitalia are extremely rare.

The *gonads* are situated intra-abdominally, in the inguinal region, or in the labia or scrotum. Bilateral ovotestes are found in a quarter of cases (bilateral form), and an ovary on one side with a testis on the other side in another quarter of cases (lateral or alternating form). In the remaining cases, an ovotestis is present on one side and either an ovary or a testis is found on the other side (unilateral form). Testicular tissue is found significantly more commonly on the right side and is usually less differentiated than the ovarian component. Mature ova develop quite frequently whereas mature spermatozoa are rarely formed. Mature follicles as well as mature spermatozoa are occasionally observed in the same patient, so that fertility or even self-fertilization is theoretically possible. Until now however, there is no known case of definite fertility.

The *internal genitalia* usually show male as well as female characteristics, and all imaginable combinations can arise. In the presence of a pure male or female gonad, the genital passages on the same side are usually of the same sexual character as the gonad. However, a Fallopian

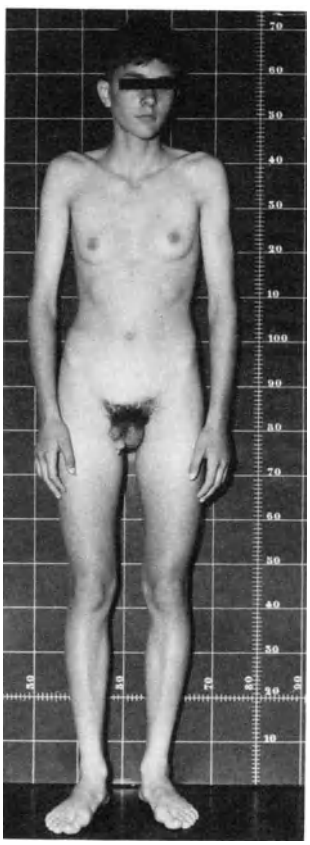


Fig. 17. 14-year-old youth with true hermaphroditism: hypospadias scrotalis (wears a post-operative urethral catheter), male stature, feminine breast development, bilateral ovotestis (left scrotal, right inguinal)

tube is usually present on the side of an ovotestis. A rudimentary or fully developed uterus is present in the majority of cases. Inguinal hernias are common.

Secondary sexual characteristics (Fig. 17) arise at the normal age and soon become more pronouncedly female or male. The course of this development cannot be predicted from the gonadal and genital findings. Female breast development arises in most cases. Menstruation occurs in about half the cases. Mentality and sexuality vary from case to case.

The *diagnosis* is not easy and is probably frequently missed. The condition must be considered in every case where external genitalia are intersexual. This is particularly applicable to chromatin-positive cases which would not be classified as nonadrenal feminine pseudohermaphroditism (p. 730) after exclusion of the adrenogenital syndrome. The true diagnosis is based on bilateral gonadal biopsies, but since biopsy from an ovotestis can reveal ovarian or testicular tissue alone under special circumstances, even bilateral biopsy is no certain method for confirming true hermaphroditism.

7. Agonadism

A special form of intersexuality termed agonadism has been described in 7 individuals brought up as girls (OVERZIER, 1956). Gonads were completely absent in these patients. No derivatives or merely rudimentary derivatives of the Wolffian and Müller's ducts were found. The external genitalia consist of a lump, similar to a somewhat enlarged clitoris. A normal urethra opens below. Other external and internal genital structures are absent. The karyotype was found to be XY in all three cases examined by one author (RATH, 1968). Involvement of siblings has been observed once (OVERZIER, 1956). Etiology and pathogenesis are not clear. According to present-day embryological knowledge (p. 708), absence of gonads should lead to female development, as is the case in Turner's syndrome. It is possible that the disturbance occurs at a much earlier stage, i.e. even before development of the Wolffian and Müller's ducts. According to OVERZIER'S theory, the "initial induction" of the gonadal "initial induction" of the gonadal "*anlage*" on the development of the genital ductile system is missing.

In order to avoid misunderstandings, it is stressed here that the terms agonadism and anorchia are used in quite different circumstances. Normal male genital development is associated with anorchia (p. 461).

F. Abnormal Genital Development in the Presence of Normal Testes (Masculine Pseudohermaphroditism)

Male pseudohermaphroditism indicates female or intersexual genital development in individuals with normal testes and usually also with a normal male XY karyotype. This is a very large group of various disorders which are compiled together in a survey presented in Table .

The pathogenesis of these disturbances is only partly explained. Fig. 18 shows once more the androgenic stimulation on genital development through the fetal testes and the nonandrogenic action of the fetal testes causing suppression of the oviduct (p. 709). If the unknown chemical factor of the fetal testes causing oviduct suppression is absent, male genital development occurs but uterus and tubes also develop at the same time. This is the syndrome of *hereditary persistence of the oviducts* (p. 725). If the periphery fails to respond to the normal androgenic stimulus of fetal testes, then female genital development occurs. Tubes and uterus are, however, not present due to the nonandrogenic action of the testes suppressing the oviducts. This gives rise to the syndrome of *testicular feminization* (p. 726). If *biosynthesis of testosterone* is disturbed in the fetal testes, then the external genitalia will be intersexual or feminine depending on the extent of the synthesis disorder, and the internal genital organs will develop normally (p. 725). Apart from these disturbances where the pathogenesis is understandable, there are numerous others whose pathogenesis is not yet known.

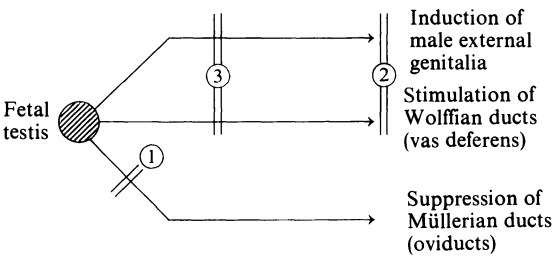


Fig. 18. Abnormal sex differentiation in male pseudohermaphroditism (XY-karyotype with normal testes). *1* Deficient suppression of oviducts: persistent oviducts. *2* Unresponsiveness to testosterone: testicular feminization. *3* Defective biosynthesis of testosterone: various enzyme defects

The situation is particularly confusing in male pseudohermaphroditism with intersexual external genitalia. Here, numerous individual cases cannot be classified satisfactorily before puberty. This is especially tragic in newborns and children, since a prognosis about the later development of puberty is impossible without a clear

classification, and this renders the decision about which sex the child should adopt more difficult. Classification in this group is based particularly on the nature of the secondary sexual characteristics and on the inheritance. *Incomplete testicular feminization* with mild masculinization of the external genitalia and female secondary sexual characteristics and the *similar syndrome with hypospadias and gynecomastia* in the presence of male secondary sexual characteristics (p. 729) are inherited through healthy women as testicular feminization (recessive X chromosome). In contrast, *hereditary vulviform perineal hypospadias* with male secondary sexual characteristics is inherited through a recessive autosomal gene. In a few cases, there is *a disturbance of the sex chromosomes* (p. 721) which can occasionally lead to quite well established testicular development and normal secondary sexual characteristics. Only the incomplete masculinization of the external genitalia and incomplete suppression of development of the uterus and tubes show that testicular development is probably not quite normal.

Finally, masculine pseudohermaphroditism with intersexual external genitalia is often found together with other malformations or as part of a dysmorphic syndrome (Table 2). Reference must be made here to pediatric textbooks on this subject.

1. Oviduct Persistence

The syndrome is also termed as internal masculine pseudohermaphroditism or as the uterine hernia syndrome. This syndrome involves mainly the absence of embryonal suppression of Müller's ducts in otherwise normal men. Either the biochemical factor concerned is absent in the fetal testes (p. 710) or Müller's ducts fail to react to it.

In known cases either unilateral or bilateral cryptorchidism is usually present and there is also a unilateral inguinal hernia. During the course of the operation, the surgeon finds a small uterus with Fallopian tubes in the abdomen or in the hernia in the presence of more or less normal testes and a normal or hypoplastic ductus deferens. The patients are otherwise unremarkable and show normal male development of the primary and secondary sexual characteristics. The degree of development of the testes depends on the position. Atrophy is often claimed to be present but normal fertility has also been confirmed in a few cases. A tumor arises in the majority of cases with intra-abdominal testes (termed teratoma, carcinoma or seminoma).

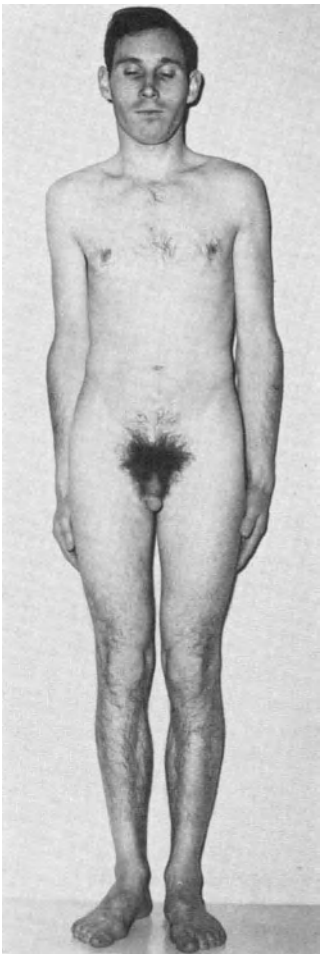


Fig. 19. 30-year-old, unobtrusive man with familiary oviduct persistence: bilateral cryptorchidism, right-sided inguinal hernia operated on at age of 18, contained uterus with tubes and testes; intra-abdominal testicular tumour (seminoma) was operated on at age 20

The diagnosis can only be made during surgical repair of cryptorchidism or during herniotomy. There are 70 recorded cases. We know of three families in which siblings were affected, which makes a sex-limited autosomal recessive inheritance appear probable.

2. Disturbances of Testosterone Synthesis

There is a series of autosomal recessive disturbances of the synthesis of steroids, of adrenocortical steroids and of testosterone formed in the Leydig cells. They can probably all be inherited. Testosterone synthesis alone may be affected, and whereas in this case genital development is uninfluenced in the girl, the deficiency of testosterone in the fetal period causes masculine pseudohermaphroditism with abnormal external and normal internal genitalia in the

boy. It also leads to reduced testosterone concentrations in the blood and urine, which cannot be stimulated by chorionic gonadotropin (HCG).

The enzyme defects so far known about are listed in Table 2 and their biochemical localization presented in Fig. 27, p. 358. Since most of the disorders are discussed fully in the chapter on the adrenal cortex, a short account is sufficient here.

In lipoid hyperplasia of the adrenals (p. 323) there is a *20-hydroxylase or other defect* which makes conversion of cholesterol into Δ5-pregnenolone impossible. The male newborn therefore suffers from an adrenal insufficiency and external genitalia are feminine or intersexual.

In the *congenital adrenogenital syndrome* with a *3β-dehydrogenase* defect (p. 371), conversion of 3-hydroxy-Δ5 steroids into 3-keto-Δ4 steroids is blocked. No glucocorticoids and no testosterone can be formed, but large amounts of dehydroepiandrosterone. This latter compound has a limited androgenic action. Adrenal insufficiency and female or intersexual external genitalia arise in the newborn boy.

Deficiency of *17-hydroxylase* (p. 359) has been little investigated in male individuals (p. 339). It blocks the synthesis of cortisol, testosterone and all other androgenic steroids. It also results in female or intersexual external genitalia in the newborn boy.

Deficiency of *17-desmolase* is the most recently described impairment of testosterone biosynthesis (ZACHMANN, 1971).

Deficiency of 17-reductase prevents conversion of androstenedione to testosterone. This defect has been seen in a male individual brought up as a girl, who had intersexual external genitalia and normal pubic hair. *In vivo*, the concentration of androstenedione in the blood was many times higher than normal and it was not possible to obtain the conversion of androstenedione to testosterone by means of excised testicular tissue.

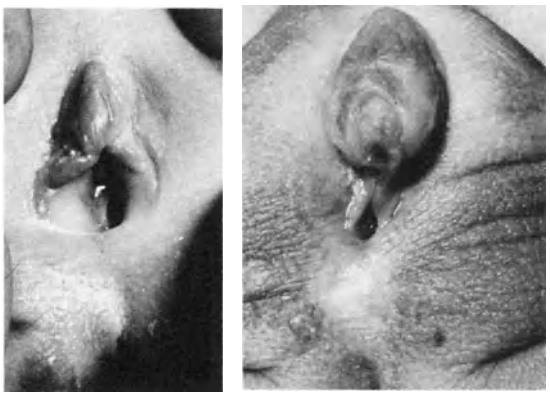


Fig. 20. Two male babies with disorders in testosterone synthesis. Left: genital Type I with lipoid hyperplasia of the adrenal cortex. Right: genital Type III with adrenogenital syndrome with 3β-dehydrogenase deficiency

3. Testicular Feminization

Testicular feminization has been known about for a long time and it is a sharply defined form of masculine pseudohermaphroditism. The term "testicular feminization" (MORRIS, 1953) is not really correct with regard to the pathogenesis, but it is precise and short. The following description would be more correct and cautious: "hereditary intersex form in the presence of an

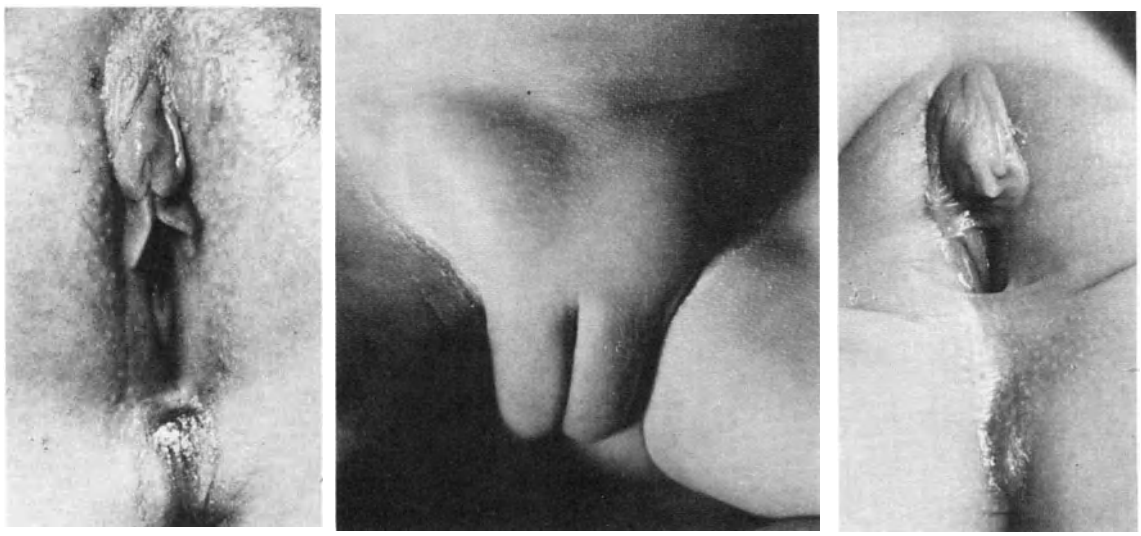


Fig. 21. External genitalia (left) and inguinal testes (middle) in testicular feminization. Right: external genitalia in incomplete testicular feminization

Fig. 22. 21-year-old woman with testicular feminization. Normal female breast development, axillary hair absent

individual of female appearance, but with male chromosomes and gonads in the absence of a uterus and sexual hair". Stressing the presence of only one symptom, WILKINS described these patients as "hairless women with testes". The frequency is estimated as 1 : 20000 to 1 : 60000.

Although the patients possess a normal male XY karyotype and testes, external genital features, breast development, voice and stature and psyche are absolutely feminine (Fig. 22), so neither the patients nor relatives doubt the sex. The feminine beauty and the well developed breasts are in fact often commented on in these patients. Usually they are tall and long-legged. Primary amenorrhea, which is always present, and sterility are *suggestive symptoms*, as well as the complete absence of pubic and axillary hair and the presence of inguinal swellings or definite inguinal hernias.

Primary amenorrhea and sterility quickly lead to recognition of the disorder after thorough gynecological examination. The external genitalia are normal or hypoplastic (Fig. 21). Breast development is normal. The sparse pubic hair, the too-short vagina ending blindly, the absence of an uterus and very often also of ovaries are striking findings. In the adult patient, small tumors which can also reach the size of a fist are found in the small pelvis or in inguinal hernias. These tumors are usually benign tubular adenomas. The vaginal smear shows normal or rather limited estrogenic effects.

Axillary hair is completely absent in the classic case. *Pubic hair* is usually present but sparsely developed.

Inguinal hernias are frequently present. They frequently arise in babies (Fig. 21) but can arise later on just as commonly. Usually they form a protrusion the size of a nut or plum above the labia majora. They occasionally give rise to pain, but mainly to vague complaints. The gonads can usually be definitely palpated. These testes are an important finding, and in infancy they are often the only indication of testicular feminization.

Hormonal findings show that testosterone levels in the blood and urine lie within normal male ranges, and that estrogen values are in the lower normal female range. Excretion of 17-ketosteroids is negligible. Levels of gonadotropins are usually slightly raised.

Examination of the internal genital organs during an exploratory operation or a herniotomy reveals the following features: uterus and tubes are absent or mere rudiments are found. The tubes may be connected with the blind-ending vagina. A rudimentary spermatic cord is occasionally found instead of a tube. The gonads are situated intra-abdominally, in the inguinal canal or very often also in the inguinal hernial sac.

Histology of the testes is exactly similar to that of undescended testes. The seminiferous tubules are usually lumenless and contain only Sertoli cells. Immature elements of spermatogenesis are rarely present, and mature spermatozoa even more rarely (KOLLER). A normal number or even an increased number of Leydig cells are present in the adult. Very often tubular adenomas which may be small or the size of a fist are also found (PICK's tubular adenoma of the testis, p. 491). The incidence of malignant tumors also tends to be high, but they are not nearly as common as in true gonadal dysgenesis and asymmetric mixed gonadal dysgenesis. The adrenals usually show no peculiarities.

The disorder is *hereditary*, is always transmitted only through healthy women and affects statistically half the male offspring. Carriers themselves also have extraordinarily poorly developed sexual hair and puberty is often claimed to have started very late. In the classic family tree (Fig. 23), siblings and aunts on the maternal side are affected, whereas the carriers (female) and a few other healthy female members of the family have poorly developed sexual hair. Since patients cannot propagate, consideration of such family trees does not exclude the possibility of an X chromosomal sex-bound recessive inheritance or an autosomal dominant mode of inheritance with sex-limited action.

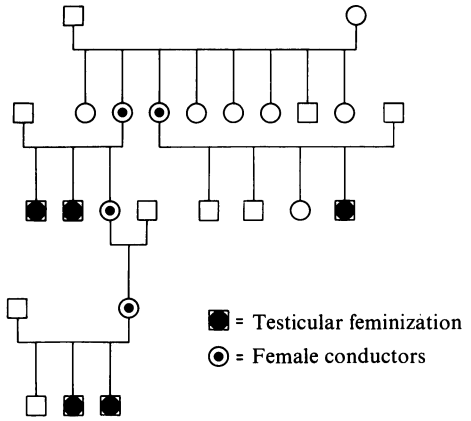


Fig. 23. Genealogical tree in testicular feminization: transmission only through healthy women. (From PRADER, 1957)

Current knowledge of the *pathogenesis* is incomplete, but there can be no doubt that the testes determine puberty of a feminine nature. In this respect, the term "testicular feminization" is justified, and is substantiated by the *effects of castration.* After surgical removal of the gonads, pronounced features of failure arise in the adult patient. Breast development regresses, the vaginal mucosa becomes atrophic, patients suffer from hot flushes, the excretion of estrogens and androgens falls and gonadotropin excretion rises.

All investigations so far indicate a *peripheral resistance to testosterone or androgens.* Testosterone production in the testes corresponds to normal male levels although the testicular tissue in vitro demonstrates a relative 3β-dehydrogenase deficiency. The effects of testosterone, however, are absent. This can be easily detected clinically and by metabolic methods. High doses of testosterone produce no signs of virilization, no hypertrophy of the clitoris, no change in voice, no increase in sexual hair or hair follicles. An anabolic effect is also absent, i.e. the usual fall in urinary excretion of phosphorous, nitrogen and citric acid fails to occur. It can therefore be presumed that resistence to androgens is already present in the fetus, resulting in suppression of male genital differentiation dependent on testosterone whereas suppression of Müller's ducts, which is independent of testosterone, occurs normally with failure of uterus and tubes to develop (p. 709, 724).

The biochemical nature of the resistence to androgens has still not been explained. It appears that testosterone can only be effective if it is first reduced to dihydrotestosterone in the tissues and that this process is inadequate in testicular feminization. This might be due to a 5α-reductase deficiency or to the increased binding capacity for testosterone in the plasma (MAUVAIS-JARVIS, 1970). The deficiency of dihydrotestosterone does not, however, offer a satisfactory explanation for the resistence to androgens, since in testicular feminization dihydrotestosterone exerts just as weak an anabolic effect as testosterone, in contrast to the effect seen in healthy subjects (STRICKLAND, 1969). Trials with rats suffering from a biologically similar hereditary disorder have given reason to believe that dihydrotestosterone is not taken up and bound by the cell nuclei (BARDIN, 1970).

The fully developed syndrome is so characteristic that the *diagnosis* can be made without the help of gonadal biopsies. In the adult, the diagnosis is made on the basis of the following main features: primary amenorrhea, abnormally sparse sexual hair, short, blind-ending vagina, negative sex chromatin associated with a woman of normal height and with normal female development of the breasts. The diagnosis is even simpler if hernias are present and if the same disorder is found in relatives on the maternal side. In children, many important symptoms are not yet present so that investigation is more difficult, but gonadal biopsy is still not essential. This diagnosis must be considered in every girl with an inguinal hernia, and the sex chromatin and the length of the vagina must be determined preoperatively.

The *differential diagnosis* includes pure gonadal dysgenesis with XY karyotype (p. 721), although in this condition secondary sexual characteristics are poorly developed and a uterus is present. Hypertrophy of the clitoris is not a sign of pure testicular feminization, but of one of the many syndromes discussed under masculine pseudohermaphroditism with intersexual genitalia (Table 2, p. 712). Differentiation is particularly important during infancy since puberty of the female type can be definitely predicted in cases of testicular feminization, whereas a masculine puberty is more likely and often almost certain in the other conditions.

There is no *treatment* for this condition. No hormone treatment can correct the amenorrhea and sterility since uterus and ovaries are missing. All that can be done is to orientate the patient or her relatives about the genital findings, prognosis (sterility) and about the heredity. In children, it is important to stress that normal female puberty will arise in spite of the absence of the uterus. The patients will naturally be looked upon as female individuals and care should be taken to avoid disclosing the gonadal and chromosomal sex. The mistake, which so commonly arises from the lack of knowledge, of declaring these children boys and masculinizing the genitalia as well as possible by surgical means has a disastrous effect with the develop-

ment of female puberty and female secondary sexual characteristics.

Bearing in mind the possibly increased risk of malignant testicular tumors, the only therapeutic problem is the question of whether the testes should be removed at the time of herniotomy and the hormone loss later replaced by long-term estrogen treatment. It is important not to remove the testes in children where this danger does not exist, but to place the testes in the abdominal cavity thus making spontaneous development of female pubertal characteristics possible. Later, one would not hesitate to remove hernias and gonadal tumors by castration and to introduce subsequent substitution therapy with female sexual hormones.

4. Incomplete Testicular Feminization and Similar Syndromes

Apart from classic testicular feminization, there is another syndrome which resembles testicular feminization in the testes and internal genital development. In this syndrome however, there is hypertrophy of the clitoris with genital Types I–III (Fig. 5), sexual hair of the female type and poor development of the breasts. Inheritance occurs through healthy women as in testicular feminization (DIEFENBACH, 1912; SCHERBAK, 1934; LUBS, 1959). The picture is always the same within a family. The term *incomplete or partial testicular feminization* seems appropriate, although no evidence of partial resistence to testosterone has so far been produced.

There are two syndromes in which male genital differentiation is somewhat more obvious and which have the same mode of inheritance. One of these is the *Gilbert-Dreyfus syndrome*, in which there is scrotal hypospadias (genital Type IV), scrotal testes, a male stature, male puberty and gynaecomastia. The other is the *Reifenstein syndrome*, characterized by hypospadias and gynaecomastia associated with an entirely male appearance, although the testes are rather small and secondary sexual characteristics are not very marked.

Testicular feminization and the two syndromes just mentioned can be considered as different stages of inadequate differentiation of the external male genitalia (FEDERMAN, 1967). The range extends from testicular feminization with complete deficiency of androgenic genital induction to the normal man with completely normal genital differentiation (Table 4). The factors common to these syndromes are inheritance through healthy mothers, absence of a true androgen deficiency despite a deficiency of androgenic effect, and almost normal suppression of Müller's ducts which is independent of androgens. It can still not be definitely said whether these are clearly definable disorders, each due to a different cause, or whether they are all *one* syndrome with varying interfamilial pronouncement and whether a gradually variable resistence to androgens is present. Resistence to androgens has so far only been demonstrated in testicular feminization.

Classification is, however, difficult in individual cases with intersexual external genitalia and where no information can be obtained about the inheritance from the family history. A pseudovaginal perineal hypospadias may also be present and in practice classification is often impossible.

5. Hereditary, Vulviform, Perineal Hypospadias

This term (also "pseudo-vaginal, perineoscrotal hypospadias") is applied to a group of individuals with intersexual external genitalia, with no uterus or tubes, and in whom normal male puberty occurs.

The genital findings can vary considerably, from a more female appearance with enlarged clitoris and genital Types II–III (Fig. 5) with abdominal testes to a more male appearance with perineal hypospadias, i.e. with intersexual

Table 4. Forms of masculine pseudohermaphroditism with varying degrees of androgenic genital differentiation, inherited through healthy mothers

	Uterus, tubes	Ductus deferens	External genitalia (Fig. 5)	Sexual hair	Breast development	Stature
Female phenotype						
– Testicular feminization	–	–	Female	–	++	Female
– Incomplete testicular feminization	–	+	I–III	+	++	Female
Male phenotype						
– Gilbert-Dreyfus syndrome	–	+	III–IV	+	+	Male
– Reifenstein syndrome	–	+	IV–V	+	+	Male
Normal male	–	+	Male	+	–	Male

genitalia of Types III–IV and scrotal or abdominal testes. In the former case, the individual is brought up to begin with as a girl, and in the latter case as a boy.

The common factor is the mode of inheritance, which in contrast to incomplete testicular feminization is not via the mother, but autosomal recessive. This is supported at least by the frequency of the condition in siblings and consanguinity of the parents when maternal aunts are not affected. The pathogenesis is unknown.

During infancy it is often impossible to differentiate between incomplete testicular feminization, which would lead to later development of female puberty, and the form discussed here, with development of male puberty, particularly if the family history gives no indication of the mode of inheritance and if the course of puberty is not helpful. It is not known either if sporadic cases with a rudimentary uterus belong here to this group. Our very inadequate knowledge of this form of masculine pseudohermaphrotitism makes it almost impossible to classify any individual case and arrive at a prognosis.

The mode of inheritance is also autosomal recessive in milder cases of hypospadias with scrotal or imperfectly descended testes. Puberty arising later is nearly always of the male type, so that surgical and psychological measures present no problems.

G. Abnormal Genital Development in the Presence of Normal Ovaries (Feminine Pseudohermaphroditism)

Feminine pseudohermaphroditism signifies male or intersexual genital development in individuals with normal ovaries and a normal female XX karyotype. The disorders conforming to this definition are grouped together in a survey presented in Table .

An abnormal effect of androgens on female genital differentiation is the most common reason for this partial to complete masculinization of the external genitalia (Fig. 24). The fetal ovaries exert no active stimulation on genital development. Genital development is always of a feminine nature in the absence of active androgenic stimulation (p. 708). An abnormal effect due to endogenous androgenic hormones of the fetal adrenal cortex is found in the *congenital adrenogenital syndrome* (p. 360). The abnormal action of exogenous hormones can be termed *transplacental virilization* (p. 731). These hormones are either androgenic or gestagenic steroids given to the mother during pregnancy or androgenic steroids secreted by ovarian or adrenocortical tumors in the mother.

The degree of masculinization of the external genitalia depends on the timing and severity of the androgenic influences. It is interesting to note that the androgenic influences are never strong enough (or never early enough) to cause development of a *ductus deferens*. Since the suppressive action exerted by the testes on the oviducts is not of an androgenic nature (p. 710), tubes and uterus develop in the normal way. Thus, only external genital development, and not internal genital development, is masculinized.

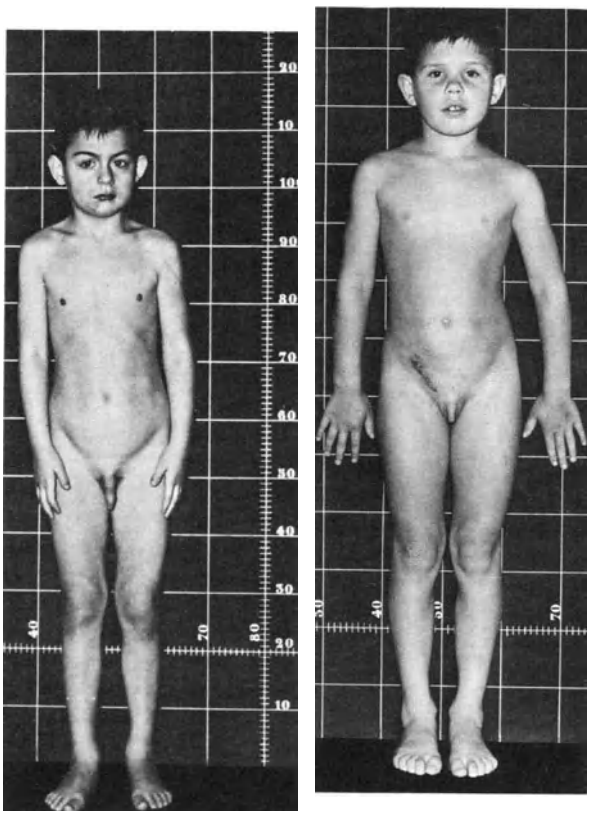


Fig. 24. Two girls with completely masculine external genitalia (genital Type V) and apparent bilateral cryptorchidism. Patient on the left; almost 5 years old, pubic hair beginning to appear in congenital adrenogenital syndrome with extreme virilization. (From PRADER, 1958.) Patient on the right: 7 years old, normal physical development and extreme transplacental virilization resulting from an androgenic adrenocortical tumour in the mother. (From MÜRSET, 1970)

The pathogenesis of these forms of feminine pseudohermaphroditism is understandable but there are cases with *complex urogenital malformations* where the pathogenesis is unknown. Some *newborns with renal agenesis* belong to

this group. Further, a special form is described where the urethra is double, consisting of vaginal and clitoric portions, is very narrow and can give rise to urinary retention.

The great majority of cases of feminine pseudohermaphroditism can be easily classified under one of the known forms. Occasionally however, cases of idiopathic hypertrophy of the clitoris or idiopathic feminine pseudohermaphroditism with normal feminine puberty can occur, but the etiology has not yet been fully explained. It is conceivable that androgenic influences from the placenta might be involved, but nothing is known about this at present.

Transplacental Virilization

If *testosterone* or *methyltestosterone* or one of the other *androgens or anabolic steroids* is given to a woman during pregnancy, the external genitalia of the newborn girl will show definite features of masculinization even when no signs of virilization are to be found in the mother. The same is true of *synthetic gestagens*, particularly ethinyl nortestosterone (nor-ethindrone) and ethinyl testosterone (etisterone) which are used in threatened abortion. *Progesterone* and *diethylstilbestrol*, which are effective in the adult, probably also have virilizing actions. Administration of large doses during the first three months of pregnancy can result in very severe Types IV–V masculinization of the external genitalia, so that the newborn may be mistaken for a boy with cryptorchidism. Milder doses or later treatment results only in hypertrophy of the clitoris. The fetus is apparently especially sensitive before and during the period of genital differentiation, and shows an androgenic reaction to steroids which produce no or hardly any androgenic response in the adult woman. The internal reproductive organs are always female. Occasionally, bone maturation is found to be advanced in newborns. Later growth and development run a normal female course appropriate to the age.

In the same manner, partial to complete masculinization of the external genitalia due to *an androgenic tumor in the mother* has been observed in a few cases. Usually an *arrhenoblastoma* is responsible, but an *androgenic adrenocortical tumor* (Fig. 24) was found in one case. The findings are exactly the same as those following administration of androgens or gestagenic steroids during pregnancy. It is extremely rare for a maternal tumor to have hormonal effects on the fetus as a virilizing tumor usually results in amenorrhea and sterility.

H. Diagnosis and Differential Diagnosis

1. Intersexual Genital Appearance

The problem of classifying the sex arises usually in the newborn, but also occasionally only later, in the presence of external intersexual genitalia. Precise investigations should follow as soon as possible after birth to put an end to the tragic uncertainty and to come to the decision which will prove most suitable in the long run. To gain time it is advisable to choose a neutral name adaptable to girl or boy (Lesley/Leslie, Christine/Christopher).

It is essential to realize that while the *genital examination* is extremely important, it is never sufficient on its own to permit a diagnosis (p. 710).

The situation is somewhat simpler in the *older child and adult* since a change of sex is no longer likely insofar as the psychosexual orientation does not definitely oppose the sex so far adopted. The different possibilities presented in Table must be kept in mind during investigation. Differential diagnosis must be based on a precise case history and thorough physical examination, with careful consideration of the following points: age at puberty (premature and of the male type in the adrenogenital syndrome), position of the gonads, absence of pubertal development (agonadism), development of male puberty (asymmetric mixed gonadal dysgenesis, hypospadias-gynecomastia syndrome, hereditary vulviform perineal hypospadias and mild forms of hypospadias, adrenogenital syndrome), development of female puberty (incomplete testicular feminization, transplacental virilization) and mixed puberty (true hermaphroditism, hypospadias-gynecomastia syndrome): amenorrhea in the presence of female puberty (incomplete testicular feminization). Important laboratory tests are: excretion of 17-ketosteroids (raised in the adrenogenital syndrome), the sex chromatin in the buccal smear and hair root and Y-fluorescent staining. The last two tests (p. 711) provide information about the number of X and Y chromosomes. When all these aspects of investigation fail to permit a definite diagnosis, exact investigation of the chromosomes and a gonadal biopsy are indicated.

Investigation is considerably more difficult in *newborns*. Here too the different possibilities given in Table 2 must be kept in mind. The family history may indicate an hereditary syndrome (various syndromes of masculine pseudohermaphroditism and the adrenogenital syndrome). An exact history of the pregnancy may suggest a transplacental virilization. The clinical state (pigmentation, salt-depletion syn-

drome) and excretion of 17-ketosteroids and pregnanetriol will lead to recognition of the adrenogenital syndrome. Sex chromatin and fluorescent staining of the Y chromosome facilitate assessment of the sex chromosomes. If an XX karyotype can be assumed then the findings of the investigations so far will permit a definite diagnosis at least as regards the possibility of a true hermaphroditism. When an XY karyotype can be assumed, abnormal gonadal development or masculine pseudohermaphroditism is probable. In these cases, examination of the chromosomes and gonadal biopsy are indicated.

2. Female Genital Appearance

In addition to the full clinical picture, there are a number of trivial and more incidental clinical findings in individuals of female appearance, which may indicate the possibility of a gonadal disorder or of masculine pseudohermaphroditism and make at least the determination of the sex chromatin desirable. A negative sex chromatin does not chance the fact that these individuals must be considered female in practical life, but it is of importance in the prognosis of growth, secondary sexual characteristics and fertility and in counseling the patient and relatives about heredity.

Turner's syndrome must be considered:

a) in all girls with unexplained dwarfism,

b) in all girls and women with pterygium colli, or any of the dysmorphic characteristics associated with TURNER's syndrome,

c) in all girls and women in whom pubic hair has developed but not the breasts,

d) in all women with primary amenorrhea.

Pure gonadal dysgenesis must also be considered when c) and d) apply.

Testicular feminization must be considered:

a) in all girls and women with inguinal hernias in which the gonads can be palpated.

b) in all women with primary amenorrhea,

c) in all women with no pubic hair.

Impaired *testosterone synthesis* must be considered in every baby of female appearance who shows signs and symptoms of the Addisonian type in the early months of life (pigmentation, anorexia, vomiting, dehydration etc.).

3. Male Genital Appearance

In these cases too there are certain features which while not striking in themselves must suggest a gonadal disorder or masculine or feminine pseudohermaphroditism. The sex chromatin at least must be determined in investigating these findings. The practical consequences of the exact diagnosis depend largely on the age of the patient (p. 733). In adult individuals they are limited to the prognosis (sterility) and a possible substitution therapy with testosterone.

Klinefelter's syndrome must be considered:

a) in every case of gynecomastia,

b) in the presence of too small testes despite visible secondary sexual characteristics,

c) in every case of male sterility.

Anorchia must be considered:

a) in every case of bilateral cryptorchidism,

b) in the absence of puberty despite a bone age of more than 13 years.

Persistence of the oviducts must be considered in men of normal male appearance with inguinal hernias and cryptorchidism.

Transplacental virilization must be considered:

a) in bilateral cryptorchidism,

b) if female puberty develops in spite of male external genitalia.

Bilateral cryptorchidism is always suggestive of a disorder of the gonads and/or of gonadal differentiation and therefore always necessitates determination of the sex chromatin. Sex chromatin is usually found in anorchia, in all forms of feminine pseudohermaphroditism, and quite frequently in Klinefelter's syndrome, in XX men, in the male Turner's syndrome, in persistence of the oviducts and in all forms of intersexual external genitalia.

I. Psychosexuality

The question of whether psyche and sexual drive in intersexuality correspond more to the genetic sex and gonadal sex or more to the nature of the genitalia and secondary sexual characteristics is one to which all investigators have always devoted a great deal of attention, and is of the utmost importance for the medical measures adopted.

The subject is widely discussed in the literature, but the information is difficult to classify and even more difficult to reduce to a common denominator. Observations about testicular feminization seem to be most consistent. It has been noticed that these genetically male individuals with testes are female not only in all physical characteristics, but also in their psyche. They are often able to have a harmonious marriage, and normal libido has often been reported. This and similar experiences have led to the view that the psychosexuality of intersexual individuals is dependent only on the character of the external genitalia and environmental influences and not on the chromo-

somes or gonads. This interpretation is applicable to some patients but there are certainly exceptions as well. It would be dangerous to make the generalization that the psychosexuality in healthy subjects was also determined only by external factors, i.e. up-bringing and environment. The most significant observations are those which reveal that the psychosexuality of numerous patients with intersexuality cannot be described simply as male or female, but is frequently poorly and inadequately developed, as in gonadal dysgenesis and in the congenital adrenogenital syndrome. It is really fundamentally wrong to ask whether the sexual drive takes a male or female trend. This explains why such patients often accept and believe in the sex allotted by their environment on the basis of their external genitalia, and why they become well adjusted even if this sex opposes the genetic and gonadal sex. The position is quite different when this natural process of adjustment is suddenly destroyed by a forcible change of sex brought about by external factors. Reasonably harmonious psychic development is hardly possible after such an event and psychoreactive disturbances, which are otherwise rare in patients with intersexuality, can then be expected. Intelligence of the intersexual lies within normal ranges.

K. Therapy and Choice of Sex

The responsibility inherent in the decision about suitable measures to be taken in the case of intersexual individuals is unusually great. The decision is based on a thorough somatic investigation and on the knowledge of psychosexuality presented in the previous section. In patients past babyhood, the decision is also based on a precise psychopathological investigation. In summary the following principles can be compiled:

1. *Individuals with purely female external genitalia* (pure gonadal dysgenesis and testicular feminization) are brought up in accordance with the genital sex. This usually occurs anyway before the diagnosis is made. Thus, the time at which the diagnosis is made does not affect the choice of sex. It would be a tragic mistake to attempt to bring up these individuals in a way corresponding to their chromosomal sex. It is best not to reveal the fact of the intersexuality, and just to discuss the prognosis of puberty and sterility. In testicular feminization, secondary sexual characteristics are of a female nature in any case. Substitution with female sexual hormones is necessary in pure gonadal dysgenesis.

2. *Investigations should be performed and the decision about further procedures made in the first year of life in all patients with intersexual external genitalia and in female patients with male external genitalia* (severe form of the adrenogenital syndrome and of transplacental virilization) in order to avoid the necessity of changing the sex later on and the associated unfavourable psychological effects as far as possible.

3. *If investigations are successfully performed in the first year or at least not later than in the second year of life*, the following measures are advisable:

a) *Girls with feminine pseudohermaphroditism and intersexual or male genitalia* are brought up according to their true sex, as girls. Female secondary sexual characteristics will develop in the adrenogenital syndrome if consistent cortisone treatment is given, and in other forms of feminine pseudohermaphroditism, female secondary characteristics will develop spontaneously. The external genitalia should be corrected surgically during the first year of life.

b) *Boys with masculine pseudohermaphroditism and intersexual genitalia and all individuals with abnormal gonads and intersexual genitalia* are brought up as belonging to the sex which corresponds best to the external genitalia. This solution must be urged since genitalia with an almost completely female appearance cannot be successfully masculinized by surgical means and since these patients, and also their parents, will have less doubts about their sex, the better the external genitalia corresponds to the assumed sex. The genitalia are corrected as much as possible by surgery, and gonads are removed if they do not correspond to the chosen sex. This appears drastic at first glance, but has no significant effect on fertility since these individuals are usually sterile, and ensures that a heterosexual puberty cannot arise later on. The parents of these castrated children must be informed that hormone therapy is essential later on for the development of pubertal characteristics.

c) To guarantee as normal a sex education as possible, the parents must be convinced from the very beginning that the correct sex has been chosen. Surgical procedures for normalization of the external genitalia should be performed as early as possible to remove any possible doubt.

4. If the case is *investigated only later*, the question of whether the sex so far accepted can still be changed under certain circumstances arises. Since sexual identification occurs during the first years of life and is settled at school age at the latest, and since any involuntary change

of sex can have unfavorable psychological effects in later life as experience has shown, there should be *no change in sex in general after the third year of life*. Even when the early decision proves wrong, it is better to stick to the sex so far assumed and to salvage the situation as best as possible by means of treatment with hormones, plastic surgery and even removal of the gonads in special circumstances. This means, for example, that a girl with feminine pseudohermaphroditism, brought up as a boy, must continue to be looked upon as a boy, and the ovaries should correspondingly be removed to prevent the development of a female puberty. An exception to this principle is made nevertheless, if a strong psychosexuality arises in definite opposition to the sex so far assumed. Such cases are, however, uncommon.

5. The following question presents a special problem: *should abdominal testes be removed prophylactically because of the increased danger of malignancy*? The danger of a malignant gonadoblastoma or dysgerminoma is so great in pure XY-gonadal dysgenesis and in asymmetric mixed gonadal dysgenesis that removal of streaks and testes must be undertaken even during infancy. The danger of malignant tumors is slight in testicular feminization, so it is better to leave the testes since they render development of puberty possible. If a hernia has to be operated on later on or a benign testicular tumor has to be removed, castration is more justified in such cases since these patients are sterile. The situation in masculine pseudohermaphroditism with intersexual genitalia of more masculine appearance is similar to that in cryptorchidism. When the testes cannot be placed in the scrotum by surgical means, removal of the testes is indicated with subsequent substitution treatment with testosterone. Later development of a tumor in abdominal testes would probably not be recognized early enough, whereas the situation can be easily detected in scrotal testes.

References

Monographs and Reviews

FEDERMAN, D.D.: Abnormal sexual development: A genetic and endocrine approach to differential diagnosis. Philadelphia and London: W.B. Saunders Co. 1967.

JIRÁSEK, J.E.: Development of the genital system and male pseudohermaphroditism, ed. by COHEN, M.M., JR. Baltimore and London: The Johns Hopkins Press 1971.

JONES, H.W., SCOTT, W.W.: Hermaphroditism, genital anomalies and related endocrine disorders. Baltimore: The Williams & Wilkins Co. 2nd ed. 1971.

LENZ, W.: Krankheiten des Urogenitalsystems. In: BECKER, P.E., Humangenetik, Bd. III/1, p. 253. Stuttgart: Georg Thieme 1964.

NEUGEBAUER, F.L.v.: Hermaphroditismus beim Menschen. Leipzig: Klinkhardt 1908.

OMBRÉDANNE, L.: Les hermaphrodites et la chirurgie. Paris: Masson & Cie. 1939.

OVERZIER, C.: Die Intersexualität. Stuttgart: Georg Thieme 1961.

— Intersexuality. New York: Academic Press 1963.

RIMOIN, D.L., SCHIMKE, R.N.: Genetic disorders of the endocrine glands. Saint Louis: C.V. Mosby Co. 1971.

WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence, 3rd ed. Springfield, Ill./USA: Ch.C. Thomas 1965.

Embryology

DÖRNER, G.: Sexualhormonabhängige Gehirndifferenzierung und Sexualität. Wien und New York: Springer 1972.

HARRIS, G.W.: Hormonal differentiation of the developing central nervous system with respect to patterns of endocrine function. Phil. Trans. Roy. Soc. B **259**, 165 (1970).

— LEVINE, S.: Sexual differentiation of the brain and its experimental control. J. Physiol. (Lond.) **181**, 379 (1965).

JOST, A.: L'analyse expérimentale de l'endocrinologie foetale. 3. Symp. Dtsch. Ges. Endocrin. Berlin-Göttingen-Heidelberg: Springer 1956.

— Embryonic sexual differentiation (morphology, physiology, abnormalities). In: JONES, H.W., SCOTT, W.W., Hermaphroditism, genital anomalies and related endocrine disorders, 2nd ed. p. 16. Baltimore: The Williams & Wilkins Co. 1971

— Désordres hormonaux et tératogénèse. Bull. schweiz. Akad. med. Wiss. **20**, 460 (1964).

— Hormonal factors in the sex differentiation of the mammalian foetus. Phil. Trans. B **259**, 119 (1970).

— A new look at the mechanisms controlling sex differentiation in mammals. Johns Hopk. med. J. **130**, 38 (1972).

LEVINE, S., MULLINS, R.F., JR.: Hormonal influences on brain organization in infant rats. Science **152**, 1585 (1966).

NEUMANN, F., ELGER, W., BERSWORDT-WALLRABE, R.v.: Intersexualität männlicher Feten und Hemmung androgenabhängiger Funktionen bei erwachsenen Tieren durch Testosteronblocker. Dtsch. med. Wschr. **92**, 360 (1967).

— — STEINBECK, H.: Antiandrogens and reproductive development. Phil. Trans. Roy. Soc. B **259**, 179 (1970).

SHORT, R.V.: The bovine freemartin: a new look at an old problem. Phil. Trans. Roy. Soc. B **259**, 141 (1970).

— SMITH, J., MANN, T., EVANS, E.P., HALLETT, J., FRYER, A., HAMERTON, J.L.: Cytogenetic and endocrine studies of a freemartin heifer and its bull co-twin. Cytogenetics **8**, 369 (1969).

Methods of Investigation

PEARSON, P.L., BORROW, M., VOSA, C.G.: Technique for identifying Y chromosomes in human interphase nuclei. Nature (Lond.) **226**, 78 (1970).

PENROSE, L.S.: Medical significance of fingerprints and related phenomena. Brit. med. J. **1968II**, 321.

PRADER, A.: Der Genitalbefund beim Pseudohermaphroditismus femininus des kongenitalen adrenogenitalen Syndroms. Morphologie, Häufigkeit, Entwicklung und Vererbung der verschiedenen Genitalformen. Helv. paediat. Acta **9**, 231 (1954).

RACE, R.R., SANGER, R.: Xg and sex-chromosome abnormalities. Brit. med. Bull. **25**, 99 (1969).

SCHMID, W.: Sex chromatin in hair roots. Cytogenetics **6**, 342 (1967).

Turner's Syndrome

ALEXANDER, D., EHRHARDT, A. A., MONEY, J.: Defective figure drawing, geometric and human, in Turner's syndrome. J. nerv. ment. Dis. **142**, 161 (1966).

BAHNER, F., SCHWARZ, G., HIENZ, H. A., WALTER, K.: Turner-Syndrom mit voll ausgebildeten sekundären Geschlechtsmerkmalen und Fertilität. Acta endocr. (Kbh.) **35**, 397 (1960).

BOEHNCKE, H., LENZ, W.: Ullrich-Turner-Syndrom bei zwei Schwestern. Z. Kinderheilk. **85**, 197 (1961).

DHADIAL, R. K., MACHIN, A. M., TAIT, S. M.: Chromosomal anomalies in spontaneously aborted human fetuses. Lancet **1970II**, 20.

FERGUSON-SMITH, M. A.: Review article: Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. J. med. Genet. **2**, 93 (1965).

— Phenotypic aspects of sex chromosome aberrations. In: Birth Defects: Original Article Series, vol. 5, 3 (1969).

FORBES, A. P., ENGEL, E.: The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis, and their close relatives. Metabolism **12**, 428 (1963).

GUPTA, D.: Steroid excretion patterns in children with gonadal dysgenesis, report of 11 cases. Clin. chim. Acta **27**, 281 (1970).

HAUSER, G. A.: Gonadendysgenesie. In: OVERZIER, C., Die Intersexualität, p. 304. Stuttgart: Georg Thieme 1961.

JOSSO, N., GROUCHY, J. DE, FREZAL, J., LAMY, M.: Familial Turner's syndrome. A study of two families with karyotypes XO and XX. Sem. Hôp. Paris (Ann. Pédiat.) **39**, 775 (1963).

KAUFMAN, S.: Vaginal bleeding in gonadal dysgenesis. Pediatrics **37**, 26 (1966).

LEMLI, L., SMITH, D. W.: The XO syndrome: A study of the differentiated phenotype in 25 patients. J. Pediat. **63**, 577 (1963).

LENZ, W., PFEIFFER, R. A., TÜNTE, W.: Chromosomenanomalien durch Überzahl (Trisomien) und Alter der Mutter. Dtsch. med. Wschr. **91**, 1262 (1966).

LINDSTEN, J., CERASI, E., LUFT, R., HULTQUIST, G.: The occurrence of abnormal insulin and growth hormone (HGH) responses to sustained hyperglycaemia in a disease with sex chromosome aberrations (Turner's syndrome). Acta endocr. (Kbh.) **56**, 107 (1967).

MEYER, R. DE: Mündliche Mitteilung (Louvain, 1970).

MORISHIMA, A., GRUMBACH, M. M.: The interrelationship of sex chromosome constitution and phenotype in the syndrome of gonadal dysgenesis and its variants. Ann. N. Y. Acad. Sci. **155**, 695 (1968).

NORA, J. J., SINHA, A. K.: Inheritance of the Turner phenotype. In: Birth Defects: Original Article Series, vol. 5, 29 (1969).

PASSARGE, E.: Gastrointestinalblutung beim Turner-Syndrom infolge Teleangiektasien in der Darmwand. Dtsch. med. Wschr. **93**, 204 (1968).

PAWLOWITZKI, I. H.: Chromosomenanomalien als Abortursache. Dtsch. med. Wschr. **91**, 1094 (1966).

POLANI, P. E.: Turner phenotype with normal sex chromosomes. In: Birth Defects: Original Article Series, vol. 5, 24 (1969).

PRADER, A.: Gonadendysgenesie und testikuläre Feminisierung. Schweiz. med. Wschr. **87**, 278 (1957).

— Die Klinik der häufigsten chromosomalen Störungen. Helv. med. Acta **29**, 403 (1962).

RACE, R. R., SANGER, R.: Xg and sex-chromosome abnormalities. Brit. med. Bull. **25**, 99 (1969).

RAINIER-POPE, C. R., CUNNINGHAM, R. D., NADAS, A. S., CRIGLER, J. F.: Cardiovascular malformations in Turner's syndrome. Pediatrics **33**, 919 (1964).

ROSSI, E.: Le tableau clinique du status Bonnevie Ullrich d'après les cas du Kinderspital de Zurich. Helv. paediat. Acta **1**, 134 (1945).

SINGH, R. P., CARR, D. H.: The anatomy and histology of XO human embryos and fetuses. Anat. Rec. **155**, 369 (1966).

TAYLOR, A. I., MOORES, E. C.: A sex chromatin survey of newborn children in two London hospitals. J. med. Genet. **4**, 258 (1967).

TURPIN, R., LEJEUNE, J., LAFOURCADE, J., CHIGOT, P. L., SALMON, C.: Présomption de monozygotisme en dépit d'un dimorphisme sexuel: sujet masculin XY et sujet neutre Haplo X. C. R. Acad. Sci. (Paris) **252**, 2945 (1961).

VALLOTTON, M. B., FORBES, A. P.: Autoimmunity in gonadal dysgenesis and Klinefelter's syndrome. Lancet **1967I**, 648.

VAN DER WERFT TEN BOSCH, J. J.: The syndrome of brachymetacarpal dwarfism ("pseudo-pseudohypoparathyroidism") with and without gonadal dysgenesis. Lancet **1959I**, 69.

WILLIAMS, E. D., ENGEL, E., FORBES, A. P.: Thyroiditis and gonadal dysgenesis. New Engl. J. Med. **270**, 805 (1964).

ZÜBLIN, W.: Chromosomale Aberrationen und Psyche. Bibl. psychiat. neurol. (Basel) No 140 (1969).

Male Syndrome of TURNER

FERRIER, P. E., FERRIER, S. A.: Turner's phenotype in the male. Pediatrics **40**, 575 (1967).

HELLER, R. H.: The Turner phenotype in the male. J. Pediat. **66**, 48 (1965).

SUMMITT, R. L.: Turner syndrome and Noonan's syndrome. J. Pediat. **74**, 155 (1969).

Pure Gonadal Dysgenesis

BARR, M. L., CARR, D. H., PLUNKETT, E. R., SOLTAN, H. C., WIENS, R. G.: Male pseudohermaphroditism and pure gonadal dysgenesis in sisters. Amer. J. Obstet. Gynec. **99**, 1047 (1967).

ELLIOTT, G. A., SANDLER, A., RABINOWITZ, D.: Gonadal dysgenesis in three sisters. J. clin. Endocr. **19**, 995 (1959).

ESPINER, E. A., VEALE, A. M. O., SANDS, V. E., FITZGERALD, P. H.: Familial syndrome of streak gonads and normal male karyotype in five phenotypic females. New Engl. J. Med. **283**, 6 (1970).

Heterosomenfragmente bei der Gonadendysgenesie. Ein Beitrag zur Beziehung Karyotyp-Phänotyp.

KARL, H. J., BACK, F., MACIAS-ALVAREZ, I., RAITH, L.: Klin. Wschr. **45**, 1225 (1967).

SOHVAL, A. R.: The syndrome of pure gonadal dysgenesis. Amer. J. Med. **38**, 615 (1965).

STERNBERG, W. H., BARCLAY, D. L., KLOEPFER, H. W.: Familial XY gonadal dysgenesis. New Engl. J. Med. **278**, 695 (1968).

SWYER, G. I. M.: Male pseudohermaphroditism: a hitherto undescribed form. Brit. med. J. **1955II**, 709.

TETER, J., BOCZKOWSKI, K.: Occurrence of tumors in dysgenetic gonads. Cancer (Philad.) **20**, 1301 (1967).

Asymmetric Mixed Gonadal Dysgenesis

AARSKOG, D.: Clinical and cytogenetic studies in hypospadias. Acta paediat. scand., Suppl. 203 (1970).

BERGADA, C., CLEVELAND, W. W., JONES, H. W., JR., WILKINS, L.: Gonadal histology in patients with male pseudohermaphroditism and atypical gonadal dysgenesis: relation to theories of sex differentiation. Acta endocr. (Kbh.) **40**, 493 (1962).

BITAN, A., DE GROUCHY, J., JOLLY, G., BACH, CH.: Dysgénésie gonadique mixte (ou différenciation gonadique asymétrique). Ann. Pédiat. **44**, 86 (1968).

FERGUSON-SMITH, M. A.: Phenotypic aspects of sex chromosome aberrations. In: Birth Defects: Original Article Series, vol. 5, 3 (1969).

FERRIER, P., FERRIER, S., KLEIN, D., FERNEX, C.: XY/XO mosaicism. Lancet **1963 I**, 54.
HANSEN, L.: XO/XY-Mosaik bei einem Knaben mit ovariell-testikulärer Dysgenesie. Dtsch. med. Wschr. **95**, 1599 (1970).
JOSSO, N., NEZELOF, C., PICON, R., DE GROUCHY, J., DRAY, F., RAPPAPORT, R.: Gonadoblastoma in gonadal dysgenesis: A report of two cases with 46, XY/45, X mosaicism. J. Pediat. **74**, 425 (1969).
NARS, P.W.: Das XO/XY-Geschlechtschromosomenmosaik. Humangenetik **7**, 185 (1969).
PFEIFFER, R.A., LAMBERTZ, B., FRIEDERISZICK, F.K., DISTEL, H., PAWLOWITZKI, J.H., NICOLE, R., OBER, K.G., RUCKES, J.: Die nosologische Stellung des XO/XY-Mosaizismus. Arch. Gynäk. **206**, 369 (1968).
SALLE, B., HEDINGER, CHR.: Gonadal histology in children with male pseudohermaphroditism and mixed gonadal dysgenesis. Acta endocr. (Kbh.) **64**, 211 (1970).
SOHVAL, A.R.: Hermaphroditism with "atypical" or "mixed" gonadal dysgenesis. Amer. J. Med. **36**, 281 (1964).

Syndrome of XX-Males

CHAPELLE, A. DE LA: Analytical review: Nature and origin of males with XX sex chromosomes. Amer. J. hum. Genet. **24**, 71 (1972).
FERGUSON-SMITH, M.A.: X-Y chromosomal interchange in the aetiology of true hermaphroditism and of XX Klinefelter's syndrome. Lancet **1966 II**, 475.

True Hermaphroditism

AARSKOG, D.: Clinical and cytogenetic studies in hypospadias. Acta paediat. scand., Suppl. 203 (1970).
BERGER, R., ABONYI, D., NODOT, A., VIALATTE, J., LEJEUNE, J.: Hermaphrodisme vrai et «garçon XX» dans une fraterie. Rev. Europ. Etudes Clin. et Biol. **15**, 330 (1970).
BUTLER, L.J., SNODGRASS, G.J.A.I., FRANCE, N.E., RUSSELL, A., SWAIN, V.A.J.: True hermaphroditism or gonadal intersexuality. Arch. Dis. Childh. **44**, 666 (1969).
JOSSO, N., DE GROUCHY, J., AUVERT, J., NEZELOF, C., JAYLE, M.F., MOULLEC, J., FREZAL, J., DE CASAUBON, A., LAMY, M.: True hermaphroditism with XX/XY mosaicism, probably due to double fertilization of the ovum. J. clin. Endocr. **25**, 114 (1965).
LANCET: True hermaphroditism (Editorial). Lancet **1967 I**, 149.
OVERZIER, C.: Intersexuality. New York: Academic Press 1963.
PRADER, A., SIEBENMANN, R.E., BETTEX, M.: Ein Fall von echtem Hermaphroditismus bei einem Kleinkind. Helv. paediat. Acta **11**, 423 (1956).
ROSENBERG, H.S., CLAYTON, G.W., HSU, T.C.: Familial true hermaphrodism. J. clin. Endocr. **23**, 203 (1963).

Agonadism

OVERZIER, C., LINDEN, H.: Echter Agonadismus (Anorchismus) bei Geschwistern. Gynaecologia (Basel) **142**, 215 (1956).
RATH, F., SCHEIBENREITER, S., THALHAMMER, O.: Agonadismus (Anorchismus) bei einem sechs Jahre alten Kind. Dtsch. med. Wschr. **93**, 633 (1968).

Persistency of Oviducts

BROOK, C.G.D., WAGNER, H., ZACHMANN, M., PRADER, A., ARMENDARES, S., FRENK, S., ALEMAN, P., NAJJAR, S.S., SLIM, M.S., GENTON, N., BOZIC, C.: Familial occurrence of persistent Müllerian structures in otherwise normal males. Brit. med. J. **1**, 771 (1973).
NILSON, O.: Hernia uteri inguinalis beim Manne. Acta chir. scand. **83**, 231 (1939).

Disturbances in the Biosynthesis of Testosterone

AARSKOG, D.: Clinical and cytogenetic studies in hypospadias. Acta paediat scand., Suppl. 203 (1970).
BONGIOVANNI, A.M.: Unusual steroid pattern in congenital adrenal hyperplasia: deficiency of 3β-hydroxy dehydrogenase. J. clin. Endocr. **21**, 860 (1961).
BRICAIRE, H., LUTON, J.P., LAUDAT, P., LEGRAND, J.C., TURPIN, G., CORVOL, P., LEMMER, M.: A new male pseudohermaphroditism associated with hypertension due to a block of 17-hydroxylation. J. clin. Endocr. **35**, 67 (1972).
CAMACHO, A.M., KOWARSKI, A., MIGEON, C.J., BROUGH, A.J.: Congenital adrenal hyperplasia due to a deficiency of one of the enzymes involved in the biosynthesis of pregnenolone. J. clin. Endocr. **28**, 153 (1968).
DEGENHART, H.J., VISSER, H.K.A., BOON, H.: A study of the cholesterol splitting enzyme system in normal adrenals and in adrenal lipoid hyperplasia. Acta paediat. scand., im Druck (abstract).
GARDNER, L.I., ASSEMANY, S.R., NEU, R.L.: 46, XY female: Anti-androgenic effect of oral contraceptive? Lancet **1970 II**, 667.
NEHER, R., KAHNT, F.W.: Gonadal steroid biosynthesis in vitro in four cases of testicular feminisation. In: Androgens in normal and pathological conditions, p. 130. Excerpta Medica Foundation 1966.
NEW, M.: Male pseudohermaphroditism due to 1α-hydroxylase deficiency. J. clin. Invest. **49**, 1930 (1970).
PRADER, A., SIEBENMANN, R.E.: Nebenniereninsuffizienz bei kongenitaler Lipoidhyperplasie der Nebennieren. Helv. paediat. Acta **12**, 569 (1957).
SAEZ, J.M., MORERA, A.M., PERETTI, E. DE, BERTRAND, J.: Further *in vivo* studies in male pseudohermaphroditism with gynecomastia due to testicular 17-ketosteroid reductase defect (compared to a case of testicular feminization). J. clin. Endocr. **34**, 598 (1972).
ZACHMANN, M.: The evaluation of testicular endocrine function before and in puberty. Effect of a single dose of human chorionic gonadotropin on urinary steroid excretion under normal and pathological conditions. Acta endocr. (Kbh.) **70**, Suppl. 164 (1972).
— VÖLLMIN, J.A., HAMILTON, W., PRADER, A.: Steroid 17, 20-desmolase deficiency: a new cause of male pseudohermaphroditism. Clin. Endocr. **1**, 369 (1972).
ZURBRÜGG, R., NEHER, R.: Steroidchemische Untersuchungen bei testikulärer Feminisierung. Ann. paediat. (Basel) **206**, 213 (1966).

Testicular Feminization

BARDIN, C.W., BULLOCK, L., SCHNEIDER, G., ALLISON, J.E., STANLEY, A.J.: Pseudohermaphrodite rat: End organ insensitivity to testosterone. Science **167**, 1136 (1970).
BOCZKOWSKI, K.: Genetical studies in testicular feminization syndrome. J. med. Genet. **5**, 181 (1968).
BULLOCK, L.P., BARDIN, C.W.: Androgen receptors in testicular feminization. J. clin. Endocr. **35**, 935 (1972).
CHARREAU, E., VILLEE, C.A.: Steroid metabolic pathways in feminizing testicular tissue. J. clin. Endocr. **28**, 1741 (1968).
FRENCH, F.S., VAN WYK, J.J., BAGGETT, B., EASTERLING, W.E., TALBERT, L.M., JOHNSTON, F.R.: Further evidence of a target organ defect in the syndrome of testicular feminization. J. clin. Endocr. **26**, 493 (1966).
GOLDSTEIN, J.L., WILSON, J.D.: Studies on the pathogenesis of the pseudohermaphroditism in the mouse with testicular feminization. J. clin. Invest. **51**, 1647 (1972).

JEFFCOATE, S. L., BROOKS, R. V., PRUNTY, F. T. G.: Secretion of androgens and oestrogens in testicular feminization: studies in vivo and in vitro in two cases. Brit. med. J. **1968 I**, 208.

JUDD, H. L., HAMILTON, C. R., BARLOW, J. J., YEN, S. S. C., KLIMAN, B.: Androgen and gonadotropin dynamics in testicular feminization syndrome. J. clin. Endocr. **34**, 229 (1972).

MAUVAIS-JARVIS, P., BERCOVICI, J. P., CREPY, O., GAUTHIER, F.: Studies on testosterone metabolism in subjects with testicular feminization syndrome. J. clin. Invest. **49**, 31 (1970).

— CREPY, O., BERCOVICI, J. P.: Further studies on the pathophysiology of testicular feminization syndrome. J. clin. Endocr. **32**, 568 (1971).

MORRIS, J. M.: The syndrome of testicular feminization in male pseudohermaphrodites. Amer. J. Obstet. Gynec. **65**, 1192 (1953).

POLANI, P. E.: Hormonal and clinical aspects of hermaphroditism and the testicular feminizing syndrome in man. Phil. Trans. B **259**, 187 (1970).

RIVAROLA, M. A., SAEZ, J. M., MEYER, W. J., KENNY, F. M., MIGEON, C. J.: Studies of androgens in the syndrome of male pseudohermaphroditism with testicular feminization. J. clin. Endocr. **27**, 371 (1967).

SCHREINER, W. E.: Über eine hereditäre Form von Pseudohermaphroditismus masculinus („testikuläre Feminisierung"). Geburtsh. u. Frauenheilk. **19**, 1110 (1959).

SIMMER, H. H., PION, R. J., DIGNAM, W. J.: Testicular feminization. Springfield, Ill./USA: Ch. C. Thomas-Publisher 1965.

STRICKLAND, A. L., FRENCH, F. S.: Absence of response to dihydrotestosterone in the syndrome of testicular feminization. J. clin. Endocr. **29**, 1284 (1969).

TREMBLAY, R. R., KOWARSKI, A., PARK, I. J., MIGEON, C. J.: Blood production rate of dihydrotestosterone in the syndrome of male pseudohermaphroditism with testicular feminization. J. clin. Endocr. **35**, 101 (1972).

Partial Testicular Feminization

BOWEN, P., LEE, C. S. N., MIGEON, C. J., KAPLAN, N. M., WHALLEY, P. S., MCKUSICK, V. A., REIFENSTEIN, E. C., JR.: Hereditary male pseudohermaphroditism with hypogonadism, hypospadias and gynecomastia. Ann. intern. Med. **62**, 252 (1965).

DIEFENBACH, H.: Familiärer Hermaphroditismus. Diss. Berlin (1912).

FEDERMAN, D. D.: Abnormal sexual development. A genetic and endocrine approach to differential diagnosis. Philadelphia and London: W. B. Saunders Co. 1967.

GILBERT-DREYFUS, S., SEBAOUN, C. A., BELAISCH, J.: Etude d'un cas familial d'androgynoidisme avec hypospadias grave, gynécomastie et hyperoestrogénie. Ann. Endocr. (Paris) **18**, 93 (1957).

LUBS, H. A., VILAR, O., BERGENSTAL, D. M.: Familial male pseudohermaphrodism with labial testes and partial feminization: Endocrine studies and genetic aspects. J. clin. Endocr. **19**, 1110 (1959).

PRADER, A.: Gonadendysgenesie und testikuläre Feminisierung. Schweiz. med. Wschr. **87**, 278 (1957).

REIFENSTEIN, E. C., JR.: Hereditary familial hypogonadism (abstract). Proc. Amer. Fed. Clin. Res. **3**, 86 (1947).

SCHERBAK, A. L.: Über die Genitalmißbildung in vier Generationen einer Familie. Wien. klin. Wschr. **47**, 432 (1934).

TETER, J., BOCZKOWSKI, K.: Testicular feminization with and without clitoral enlargement. Amer. J. Obstet. Gynec. **94**, 813 (1966).

Hereditary Vulviform Perineal Hypopsadia

DRASH, A., SHERMAN, F., HARTMANN, W. H., BLIZZARD, R. M.: A syndrome of pseudohermaphroditism, Wilms' tumor, hypertension, and degenerative renal disease. J. Pediat. **76**, 585 (1970).

LENZ, W.: Krankheiten des Urogenitalsystems. In: BECKER, P. E., Humangenetik, Bd. III/1, p. 253. Stuttgart: Georg Thieme 1964.

SØRENSEN, H. R.: Hypospadias. With special reference to aetiology. Copenhagen: E. Munksgaard 1953.

VAAL, O. M. DE: Genital intersexuality in three brothers, connected with consanguineous marriage in the three previous generations. Acta paediat. **44**, 35 (1955).

Pseudohermaphroditismus femininus

CARPENTIER, P. J., POTTER, E. L.: Nuclear sex and genital malformation in 48 cases of renal agenesis, with especial reference to nonspecific female pseudohermaphroditism. Amer. J. Obstet. Gynec. **78**, 235 (1959).

HOWARD, F., HINMAN, F.: Female pseudohermaphroditism with supplementary phallic urethra: report of two cases. J. Urol. (Baltimore) **65**, 439 (1951).

SCHLEGEL, R. J., ASPILLAGE, M. J., NEU, R. L., CARNEIROLEAO, J., GARDNER, L. I.: An XX sex chromosome complement in an infant having male-type external genitals, renal agenesis, and other anomalies. J. Pediat. **69**, 812 (1966).

SERINGE, PH., BACH, CH., LOEWE-LYON, S., HALLEZ, J., BOCQUET, L., VERON, P.: Les différentes variétés de pseudohermaphrodisme féminin ne relevant pas de l'hyperplasie surrénale congénitale. Sem. Hôp. (Paris) **37**, 57 (1961).

Transplacentar Virilization

BONGIOVANNI, A. M., DIGEORGE, A. M., GRUMBACH, M. M.: Masculinization of the female infant associated with estrogenic therapy alone during gestation: four cases. J. clin. Endocr. **19**, 1004 (1959).

GRUMBACH, M. M., DUCHARME, J. R., MOLOSHOK, R. E.: On the fetal masculinizing action of certain oral progestins. J. clin. Endocr. **19**, 1369 (1959).

MÜRSET, G., ZACHMANN, M., PRADER, A., FISCHER, J., LABHART, A.: Male external genitalia of a girl caused by a virilizing adrenal tumour in the mother. Acta endocr. (Kbh.) **65**, 627 (1970).

OVERZIER, C.: Induzierter Pseudohermaphroditismus. In: OVERZIER, C., Die Intersexualität, S. 394. Stuttgart: Georg Thieme 1961.

WILKINS, L., JONES, H. W., HOLMAN, G. H., STEMPFEL, R. S.: Masculinization of the female fetus associated with administration of oral and intramuscular progestins during gestation: Non-adrenal female pseudohermaphrodism. J. clin. Endocr. **18**, 559 (1958).

Psychosexuality and Assignment of Sex

DEWHURST, C. J., GORDON, R. R.: Change of sex. Lancet **1963 II**, 1213.

MONEY, J.: Psychologic evaluation of the child with intersex problems. Pediatrics **36**, 51 (1965).

— EHRHARDT, A.: Psychosexual differentiation. Baltimore: Johns Hopkins Press (in press).

TETER, J., BOCZKOWSKI, K.: Errors in management and assignment of sex in patients with abnormal sexual differentiation. Amer. J. Obstet. Gynec. **93**, 1084 (1965).

ZÜBLIN, W.: Chromosomale Aberrationen und Psyche. Bibl. psychiat. neurol. (Basel) No 140 (1969).

XIII. The Pancreas

G. R. CONSTAM

With Contributions by

E. R. FROESCH, CHR. HEDINGER, G. KISTLER, P. H. ROSSIER, H. STEINER, and G. TÖNDURY

A. Historical Dates

G. R. CONSTAM

Around 1500 B. C., Papyrus Ebers, Egypt; description of abnormal polyuria, possibly diabetes mellitus.

6th century B. C. The Indians differentiated the asthenic from the sthenic form of diabetes mellitus. In the Ayur Veda of Susruta, the illness is termed as "madhumeha" or "honey-urine".

A few centuries B. C., the Chinese recognized the sweet taste of the urine.

30 B. C. to 50 A. D. AULUS CORNELIUS CELSUS described a condition in which much urine was excreted.

30 to 90 A. D. ARETAEUS OF CAPPADOCIA gave the same description as CELSUS and called it "diabetes".

131–201 A. D., GALEN described diabetes as a weakness of the kidneys.

860–932 RHAZES, an Arabian physician, discussed the treatment of diabetes mellitus.

980–1027 AVICENNA believed that the liver was particularly affected in diabetes, and observed the connection between diabetes, furunculosis and impotence.

1621–75 THOMAS WILLIS, in Oxford, distinguished diabetes mellitus from diabetes insipidus, and showed that the urinary sugar was increased in the former condition.

1682 JOHANN CONRAD BRUNNER observed polyuria and polydipsia in a dog after removal of the pancreas.

1774 ROBERT WYATT suspected the presence of a substance similar to sugar in the urine and blood. He obtained this substance by evaporating the urine.

1776 DOBSON demonstrated a fermentable sugar in the urine and the sweet taste of the blood of diabetic patients.

1788 THOMAS CAWLEY suspected a connection between diabetes and changes in the pancreas.

1796 ROLLO recommended a low-calory diet in the treatment of diabetes and described the smell of acetone.

1815 CHEVREUL identified the sugar in diabetes as glucose.

1806–86 BOUCHARDAT utilized fermentation tests, the polarimeter and solutions of copper salts, for estimating sugar. He substituted fat and alcohol for carbohydrates, emphasized the value of green vegetables, of a low calory diet, and of much physical activity. He introduced days of fasting and the use of alkali, and discovered gluten bread.

1848 HERMANN VON FEHLING described the urine test which was later named after him.

1849 CLAUDE BERNARD discovered glycogen in the liver, and the "piqûre". He made quantitative estimations of the sugar in the blood.

1869 PAUL LANGERHANS discovered the islet cells of the pancreas.

1882 CHAUFFARD and HANOT described the combination of pigment-cirrhosis and diabetes as "bronze diabetes".

1889 V. RECKLINGHAUSEN revealed the nature of the two pigments of "bronze diabetes", and introduced the term hemochromatosis.

1889 O. MINKOWSKI and J. VON MERING incidentally discovered that total pancreatectomy in a suitable experimental animal produces diabetes.

1891 GIULIO VASSALE ligated the excretory ducts of the pancreas, which led to the destruction of the acini, but not of the islet cells.

1892 O. MINKOWSKI produced temporary disappearance of diabetes in dogs by subcutaneous implantation of the excised pancreas.

1893 LAGUESSE suspected that the islet cells formed a hormone.

1895 V. NOORDEN developed a technique of dietary therapy, stressed the formation of sugar from protein, and introduced the course of oats as a treatment.

1898–1962 E. P. JOSLIN untiringly improved the treatment of diabetes mellitus.

1906 NAUNYN studied the metabolism in diabetes, particularly in diabetic acidosis. He emphasized the familial occurrence

of the disease, and the value of a just adequate nourishment in the prophylaxis and in the treatment of the metabolic disturbance.

1908 ZUELZER gained an alcoholic extract from the pancreas, which after being injected, produced shock – probably of hypoglycemic nature – causing the trial to be discontinued.

1909 DE MEYER gave the name insulin to the still hypothetical hormone of the islet cells.

1913 F. M. ALLEN became famous for his hunger cures. He also contributed to the knowledge of carbohydrate metabolism.

1918 C. K. WATANABE produced hypoglycemia in the animal with an injection of guanidine.

1921 N. C. PAULESCO in Rumania reported "pancréine", i.e. a blood sugar-lowering extract from pancreas of dogs or cattle, which he had discovered during the First World War (1914–18).

1921 FREDERICK G. BANTING and CHARLES H. BEST discovered insulin.
F. C. MANN and T. B. MAGATH showed that hepatectomy results in hypoglycemia.

1924 B. A. HOUSSAY and MAGENTA noticed that hypophysectomy increases sensitivity to insulin.

1924 SEAL HARRIS suspected hyperinsulinism as a cause of spontaneous hypoglycemia.

1926 E. FRANK, M. NOTHMANN, and A. WAGNER introduced biguanidines into the treatment of diabetes which, however, was abandoned in 1940.
ABEL succeeded in crystallizing insulin.

1927 WILDER, ALLAN, POWER, and ROBERTSON published the first case of organic hyperinsulinism.

1929 HOWLAND, CAMPELL, MALTBY, and ROBINSON removed an islet-cell tumor and cured a case of hyperinsulinism for the first time.

1936 H. C. HAGEDORN produced the first reliable insulin with prolonged action.

1937 F. G. YOUNG discovered meta-pituitary diabetes.
H. R. JACOBS observed alloxan-hyperglycemia.

1942 GUEST pointed out hypokalemia during the treatment of diabetic acidosis.

1942 M. JANBON noticed the hypoglycemic action of one of the sulfonamides recommended for the treatment of typhoid fever.

1943 DUNN, SHEEHAN, and MACLETCHIE discovered alloxan-diabetes.

1944 A. LOUBATIÈRES explained the mode of action of certain hypoglycemic agents.

1955 H. FRANKE and J. FUCHS observed hypoglycemia produced by another sulfonamide, and suggested that it should be used therapeutically in diabetes mellitus.

1955 F. SANGER discovered the structural formula of the insulin molecule.

1957 G. UNGER introduced phenethyl biguanide into the treatment of diabetes.

1957 S. A. BERSON and R. S. YALLOW measured the insulin content of the plasma by radioimmunological methods.

1964 H. ZAHN in Germany, KATSOYANNIS in U.S.A, and NIU CHING-I in China (1965), all independently succeeded in synthesizing insulin.

1967 D. F. STEINER and P. OYER isolated proinsulin.

1969 Mrs. D. G. HODGKIN discovered the three-dimensional structure of pig insulin.

B. Embryology and Histology

G. TÖNDURY and G. KISTLER

In the human embryo 3 to 4 mm in length, two entodermal outpocketings arise on opposite sides of the primitive duodenum. One of the epithelial buds grows out from the dorsal wall of the gut, just above the hepatic diverticulum; it forms the *dorsal* pancreas. The *ventral* pancreas, on the other hand, originates in the caudal angle between hepatic diverticulum and gut. When the embryo is about 12 mm long, the two primordia meet, and when it is about 16 mm, they fuse to produce a joint organ. With the exception of the major parts of the head and the uncinate process, which are derivatives of the ventral bud, most of the mature gland is formed by the dorsal pancreas anlage. Both primordia are crossed by an axial longitudinal duct. The duct of the dorsal anlage originates directly from the wall of the duodenum, whereas the ventral duct opens into the stem of the elongating common bile duct. When duodenal torsion has brought the two primordia into close side-by-side contact, the ventral duct taps its dorsal counterpart. The major pancreatic duct of the mature gland (duct of Wirsung) results, therefore, from the fusion of the ventral duct with the distal segment of the dorsal duct. The proximal stem segment of the dorsal duct, on the other hand, constitutes the accessory duct of Santorini, which usually retains its connection to the duodenum as well.

The islets of Langerhans develop from epithelial cells of the outgrowing pancreatic ducts. They are, therefore, of entodermal origin. Even

in the embryo of 18 mm in length (age about 7 weeks), the terminal and side buds of the primitive ducts contain a few granular cells which can be selectively blackened by silver-salt solutions. These cells multiply and form single solid sprouts which enlarge to become the fetal (and early post-natal) islets. These so-called primary islets consist of a central mass of insulin-producing type-B cells which are surrounded by a compact layer of glucagon-synthesizing type-A cells (FERNER, 1952). Between these two subdivisions, a transitional zone containing agranular cells soon develops. Here, type-A cells appear to be transformed continuously into type-B cells. The core of the islet thus enlarges considerably and the islet itself resembles more and more the one found in the adult pancreas.

The mature islets of Langerhans are rounded or ovoid epithelial complexes usually situated

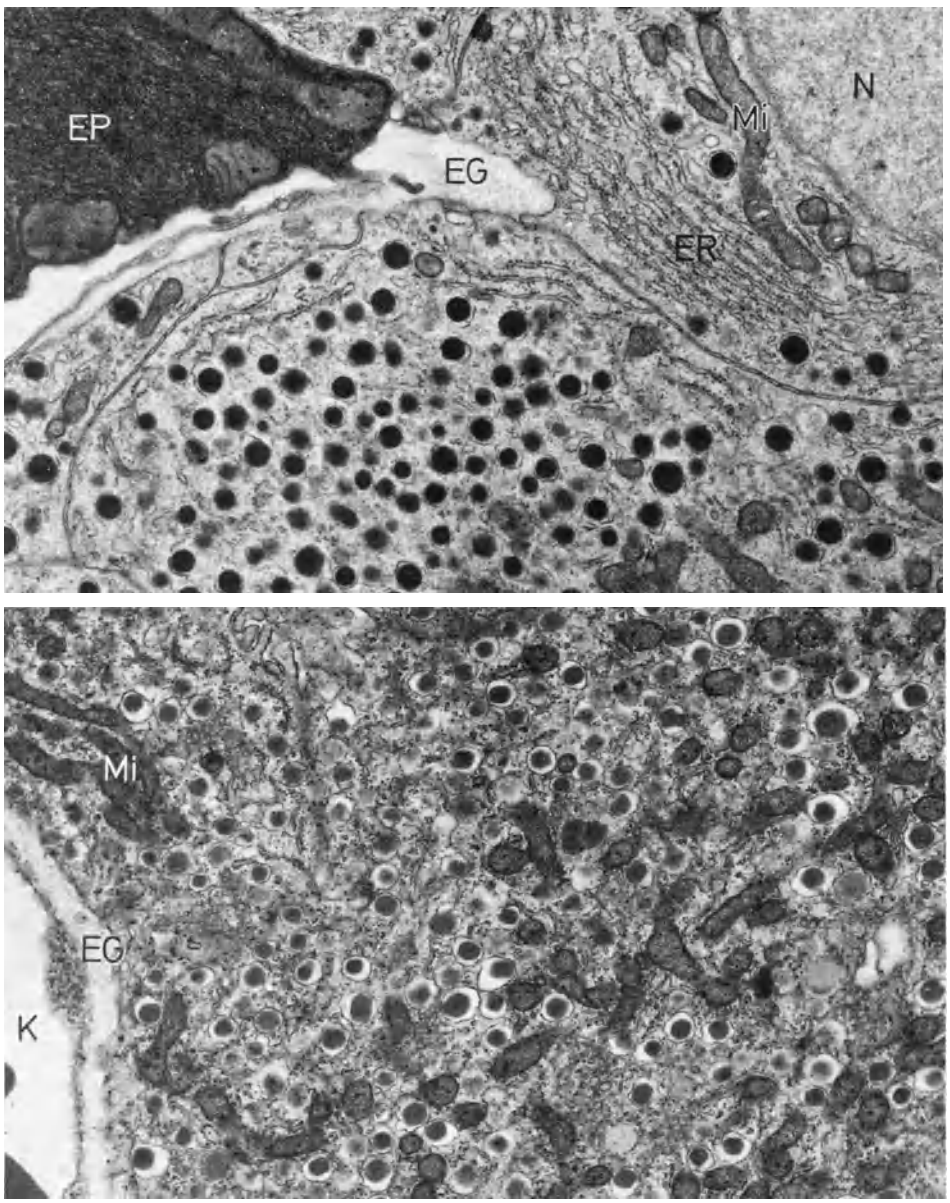


Fig. 1a and b. The granules of the glucagon-producing A-cells are electron-dense and separated from the surrounding membrane by a narrow, clear zone. In contrast, the often eccentrically located content of the granules of the insulin-synthesizing B-cells (lower half of the picture), usually display a much wider halo. Both types of granules are scattered throughout the cytoplasmic ground substance, but tend to be more numerous in the cell portions contacting the capillaries. *EP* exocrine pancreas; *K* capillary; *N* nucleus; *ER* rough-surfaced endoplasmic reticulum; *Mi* mitochondria; *EG* extravascular, interstitial space containing fibroblast processes as well as collagenous fibrils. × 12000

in the central portions of the lobules of the exocrine pancreas. They may also, however, be found in the interlobular connective tissue or associated with the smaller branches of the pancreatic ducts. Although the islets are scattered throughout the exocrine pancreatic parenchyma, they are more numerous in the tail than in the head or body of the gland.

The highly vascularized islets receive their blood supply through branches of the interlobular arteries which arise from both the celiac trunk and the superior mesenteric artery. The thin capsule of reticular fibers separating the islets from the exocrine parenchyma is usually penetrated by one arteriole. Within the islet, this arteriole splits up into a number of anastomosing capillary loops which are in intimate contact with the epithelial cords, like the vas afferens in a kidney glomerulum. The endothelium of these capillaries varies considerably in thickness. In attenuated portions of the endothelial cells, typical fenestrae (endothelial pores) are present. A few vasa efferentia convey the blood into the interlobular venules of the exocrine pancreas.

The functional significance of the unmyelinated nerve fibers found to penetrate into the islets is still a matter of dispute. In various mammalian species, the nerve fiber terminals found in close proximity to the A-type cells contain synaptic vesicles which differ morphologically from those observed in axon endings contacting B-type cells. It is postulated that the innervation of the A-cells is cholinergic, whereas that of the B-cells is adrenergic (WATARI, 1968).

Light microscopy reveals that the islet cells are less stainable than the acinar cells of the exocrine pancreas. In hematoxylin and eosin preparations, the cytoplasm of these cells appears more or less homogeneous, but with the Mallory-azan stain, three types of cells can be easily distinguished, all of them displaying distinct granules in their cytoplasm. The *A-cells*, which are found predominantly at the periphery of the islets, contain granules which stain red and are insoluble in alcohol. The somewhat smaller granules of the more centrally located *B-cells*, on the other hand, stain orange and are dissolved in alcohol. A third cell type, the so-called *D-cell*, is scattered throughout the islet. It displays granules which stain blue. In some mammalian species, a fourth, *agranular* cell type has been described. It is usually referred to as the *C-cell*.

Electron microscopy shows that the A- (or alpha) cells contain large numbers of membrane-bound, electron-dense granules. Characteristically, the granule is separated from its membrane by a very narrow, electron-translucent cleft (Fig. 1a). A few profiles of rough-surfaced endoplasmic reticulum, and rather small, ovoid or elongated mitochondria are scattered throughout the cytoplasmic ground substance. The inconspicuous Golgi apparatus is situated in a juxtanuclear position. The size and electron density of the granules of the B-cells (beta cells) vary considerably in the different mammalian species. The clear zone separating the often eccentrically-located granulum from its membrane is usually much wider than in the A-granules (Fig. 1b). In the human B-cells, some of the granules contain rounded or polygonal cristalloid structures which display an internal periodicity. The mitochondria of this cell type appear to be more numerous than in the A-cells. The Golgi complex is well developed. Between the granules, elongated and vesicular profiles of the rough-surfaced endoplasmic reticulum are intermingled with free ribosomes and small vesicles. The granules of the D-cells (delta cells) are larger and much less electron-dense than those of the A- and B-cells. The functional significance of this cell type is still a matter of dispute. The presence, in some mammalian species, of A- and D-type granules within the same cell, would seem to corroborate the assumption that the D-cell (delta cell) is an altered A-cell. However, D-cells are also present in young individuals and lack degenerative changes in the nucleus and cytoplasm.

C. The Islet Cell Apparatus, Metabolic Regulation, and Pathophysiology of Diabetes Mellitus

E. R. FROESCH

Diabetes mellitus is the most common endocrine disease. Contrary to the majority of the hyper- and hypofunctional endocrine syndromes, diabetes mellitus ist one of GARROD'S "inborn errors of metabolism" (STANBURY, 1966). But even among these diseases, diabetes mellitus is peculiar in that the disturbance of carbohydrate metabolism, glucose intolerance, is usually not congenital, but rather becomes apparent later in life. The lack of insulin is, therefore, not congenital in most cases, but develops sooner or later during life. The current opinion is that insulin deficiency is not due to a single genetic defect and that exogenous factors accelerate the manifestation of underlying condition as diabetes mellitus. In order to understand diabetes mellitus as a disease, and its management and therapy, an elementary knowledge of the important metabolic processes and their control is essential.

1. Energy Exchange and Intermediary Metabolism

Every living cell has a basal energy requirement, i.e. it needs fuel and oxygen. Oxidation of substrates helps to maintain a constant temperature and yields the energy for synthetic processes occurring constantly in the organism. In poikilothermic animals, the basal metabolic rate varies according to the outside temperature. This is a result of the temperature-dependent enzyme reactions. All enzymes have an optimum temperature and, in general, operate more slowly at lower temperatures. At low temperatures, breakdown and turnover of ribonucleic acids and desoxyribonucleic acids are slowed, and so are protein and enzyme synthesis, resulting in a fall of the basal energy requirement. In the warm blooded, the situation is reversed and the body temperature is maintained at a constant level. At lower temperatures the heat loss, and thus heat production and energy requirements, increase.

The energy obtained through the oxidation of substrates is not only converted to heat but is stored in the form of energy-rich phosphate The energy reserves may then be used for biosynthetic processes or for muscle contraction.

Adenosine triphosphoric acid (ATP) is the most important energy-rich co-enzyme and it is formed during the process of oxidative phosphorylation. ATP transfers energy-rich phosphate onto glucose with the help of hexokinase or glucokinase, thus converting glucose to glucose-6-phosphate, a compound richer in energy and more reactive than glucose.

In muscle, creatine phosphate is another important energy-rich coenzyme. During muscular exercise, creatine phosphate gives off its phosphate to adenosine diphosphate to form ATP, providing the energy for muscle contraction. The creatine liberated is either broken down to creatinine and excreted in the urine, or phosphorylated with ATP to form creatine phosphate again during muscle rest.

Different metabolic pathways are open to glucose-6-phosphate. When substrate is abundant, it is converted to glucose-1-phosphate and glycogen by the insulin-sensitive glycogen-synthetase. Glycogen is then stored (LARNER, 1964; LELOIR, 1964). The storage of glycogen in liver and muscle is limited, in contrast to the storage of fat in adipose tissue.

Maximum glycogen reserves of the body amount to 200–400 g (800 to 1600 calories) and cover the energy requirement for one day at the most.

The conversion of glucose to fat is a complicated process. Glucose-6-phosphate goes all the way through glycolysis down to pyruvate, which is decarboxylated to acetyl-coenzyme A. Two molecules of acetyl-Co A condense to form acetoacetyl-Co A (mitochondrial synthesis of fatty acids) or else, acetyl-Co A fixes CO_2 and forms malonyl-Co A (cytoplasmic synthesis of fatty acids) (LYNEN, 1967). The chains become longer through further condensation with acetyl-Co A and are then reduced to fatty acids by triphosphopyridine nucleotide ($NADPH^+$) and diphosphopyridine nucleotide ($NADH^+$). Fatty acids are then esterified with alphaglycerophosphate to phosphatidic acid and finally to triglycerides, in which form they are stored. The $NADPH^+$ needed for the reductive synthesis of fatty acids is formed predominantly in the pentose-phosphate shunt, whereas $NADH^+$ arises mainly in the tricarboxylic-acid cycle. Fatty-acid synthesis corresponds to the activity of the pentose-phosphate shunt, from which the essential reducing potential is derived.

During anaerobic glycolysis 1 molecule of glucose and 2 molecules of ATP produce 2 molecules of lactic acid and 4 molecules of ATP. During glycolysis 2 molecules of ATP are gained from every molecule of glucose. However, only a small part of the available energy of the glucose molecule is liberated in this way. During complete oxidation of glucose to H_2O and CO_2 in the tricarboxylic-acid cycle, a further 30 ADP molecules are phosphorylated to ATP. The oxidation of the substrates is coupled with the formation of energy-rich ATP. For each molecule of oxygen 3 molecules of ATP are formed. The biologic oxidation becomes a highly efficient combustion machine as a result of this coupling of oxidation to the formation of ATP as an energy store. Approximately 40–50% of the energy liberated during the biological combustion of glucose and fatty acids are not released as heat but rather used for synthesis.

a) Regulation of Energy Exchange

The energy requirement of the muscle is not related to the substrate supply. It depends primarily on muscular work. The uptake of substrate and oxygen from the blood is, therefore, dependent on the work performed. It is in part controlled by hormones during the phase of rest and storage. During acute muscular activity, ATP is used and is later replaced by ATP generated during glycolysis. The musculature extracts free fatty acids and glucose from blood as an energy source during prolonged excercise.

We now face the complex question of how the uptake of combustible substances by different tissues is regulated. Two major factors come into play: 1. the need of the tissue for com-

bustible substances to cover its energy requirement, 2. the supply of combustible substances.

The local needs of a certain tissue for substrate cannot be controlled by hormones which act indiscriminately on all tissues. A working muscle, for example, must itself be capable of adjusting to its elevated energy requirement during work by an increased uptake of substrate (SANDER, 1964). Therefore, two fundamentally different regulations for the uptake of glucose by muscle must exist. One is controlled by insulin and affects glucose transport. The other is controlled intracellularly through stimulation of certain enzyme reactions.

b) Mechanism of the Insulin-Independent Uptake of Glucose by the Muscle during Work (compare Fig. 2)

Glycolysis is regulated by three processes:

1. Through the activity of phosphorylase which splits off the terminal glucose molecule of the glycogen chain by phosphorylating them with inorganic phosphorus to form glucose-1-phosphate (ILLINGWORTH, 1964);

2. through the transport of glucose through the cell membrane, and

3. through the activity of two key enzymes, hexokinase and phosphofructokinase, which perform irreversible reactions which are inhibited by their end products (HELMREICH, 1964).

1. Glucose + ATP $\xrightarrow[Mg^{++}]{\text{Hexokinase}}$ Glucose-6-phosphate + ADP.

2. Fructose-6-phosphate + ATP $\xrightarrow[Mg^{++}]{\text{Phosphofructokinase}}$ Fructose-1,6-diphosphate + ADP.

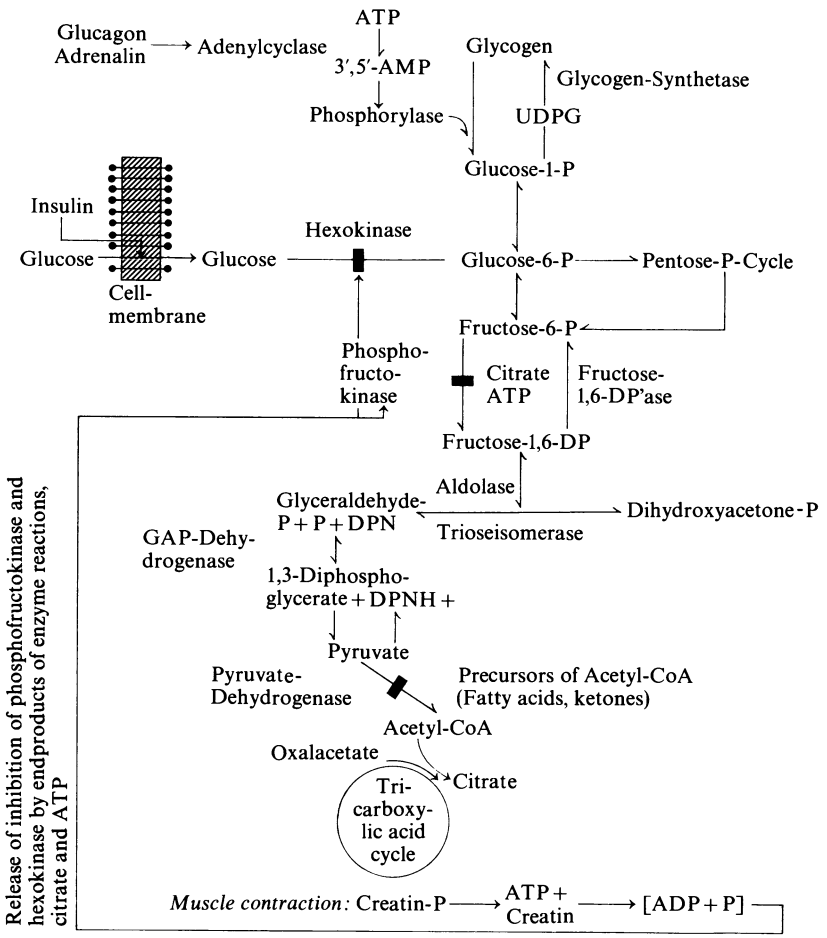


Fig. 2. The phosphorylating enzymes, glucokinase, hexokinase and phosphofructokinase are the key enzymes in glycolysis. Hexokinase and phosphofructokinase are inhibited by their enzymatic end products. Fatty acids and ketone bodies inhibit pyruvate dehydrogenase and the entire glycolysis by their oxidation to acetyl-coenzyme A and competition for coenzyme A. Apart from this, citrate accumulates and causes an inhibition of phosphofructokinase. During muscular contraction, ADP and inorganic phosphate arise from energy-rich ATP. Both products help to prevent substrate inhibition of hexokinase and phosphofructokinase and lead to increased glucosephosphorylation and glucose oxidation

Phosphorylase occurs in an active (a) and an inactive (b) form (KREBS, 1964). Phosphorylase-kinase converts phosphorylase from the b-form into the a-form. K^+ and Ca^{++} ions and cyclic 3′,5′-adenosine monophosphate catalyze this process. However, the exact mechanism by which electrical stimulation and the contraction of muscle fiber activate phosphorylase, thereby making glycogen available for anaerobic glycolysis, remains to be explained (DANFORTH, 1965; HELMREICH, 1965; BUEDING, 1965).

The accumulation of fructose-1,6-diphosphate has a contrary effect, leading to an increase of glucose-6-phosphate concentration and thus to an inhibition of glucose phosphorylation. Inorganic phosphate seems to counteract this inhibition so that glycolysis is resumed. It may be assumed that among other factors, muscular activity stimulates glycolysis through an elevated level of intracellular inorganic phosphate. During muscular contraction, ATP supplies the energy and is converted to ADP and inorganic phosphate. The increase in inorganic phosphate would then prevent substrate inhibition of phosphofructokinase and re-establish glycolysis (UYEDA, 1965).

Insulin promotes the transport of glucose through the cell membrane and determines how much glucose is taken up and stored as glycogen and triglycerides in tissues. Compared to the effect of insulin, the inhibition of glycolysis by fatty acids is probably only of minor physiologic and pathophysiologic significance.

When glucose turnover is increased through muscular activity or during anoxia (Pasteur effect), glucose is also transported into the cells by so-called "facilitated diffusion". The relationship between energy exchange of the cells and activation of this transport process has not been explained.

The uptake of glucose by muscle is inhibited by the simultaneous uptake of other substrates, in particular by fatty acids, ketone bodies and pyruvic acid (OPIE, 1963; RANDLE, 1964; WILLIAMSON, 1965). Two mechanisms are important: The ratio of acetyl-coenzyme A to coenzyme A increases with a rising supply of fatty acids and ketone bodies. Pyruvate dehydrogenase, which converts pyruvate to acetic acid, is inhibited. In addition, the citrate concentration increases and inhibits phosphofructokinase.

The different factors which influence the utilization of glucose by muscle not directly through the membrane-transport system, but rather through a step in the intermediary metabolism, are still being discussed. A detailed discussion of this problem would lead too far. We still do not know whether the excessive supply of substances such as fatty acids which inhibit glucose uptake by muscle, is causally connected with glucose intolerance (see Fig. 2).

c) Free Fatty Acids as a Source of Energy for Muscle

Glucose supplies only a small part of the energy required by resting and exercising muscle. Free fatty acids and ketones are the main source of energy. They are released into the blood from adipose tissue after hydrolysis of stored triglycerides (lipolysis). The oxidation of glucose, fatty acids and ketones increases in muscle during work (INGLE, 1955; HAVEL, 1963). Glucose contributes only 10–15% and free fatty acids and ketones 70–90% to the total energy expenditure of excercising muscle (PAUL, 1967).

d) Regulation of Glucose Transport through the Cell Membrane

The membranes of most cells are impermeable to glucose. Glucose does not diffuse freely into the cells. Erythrocytes and parenchymal liver cells contain free glucose. Glucose is transported into the cells by a carrier mechanism not requiring energy (facilitated diffusion) (WILBRANDT, 1961). This mechanism of facilitated diffusion differs in principle from pumping transport mechanisms by which a concentration gradient may be overcome, as for instance during glucose absorption in the gut or renal tubular reabsorption of glucose. These active transport pumps necessitate energy for functioning. They are coupled in some form to ATP-ases which supply the necessary energy for the transport against a concentration gradient by splitting energy-rich ATP (CRANE, 1960).

The transport of glucose is the limiting step of total glucose utilization by muscle and adipose tissue, the two major tissues which are sensitive to insulin (FROESCH, 1962; CROFFORD, 1965; MORGAN, 1961). Teleologically this makes good sense. The transport process can be increased instantaneously by insulin. Excessive amounts of enzymes in the cells are ready to phosphorylate and oxidize glucose or to store it in the form of glycogen or fat, depending on the tissue and the needs. Insulin exerts other actions on the cells not related to transport. Its effect on transport is certainly the most important. Stimulation of enzyme synthesis would necessitate too much time to ascertain glucose homeostasis.

2. Glucose Metabolism in the Liver

Glycogenolysis, Gluconeogenesis and their Regulation (see Fig. 3)

The daily calorie requirement of man at rest lies around 25–30 calories per kilogram of

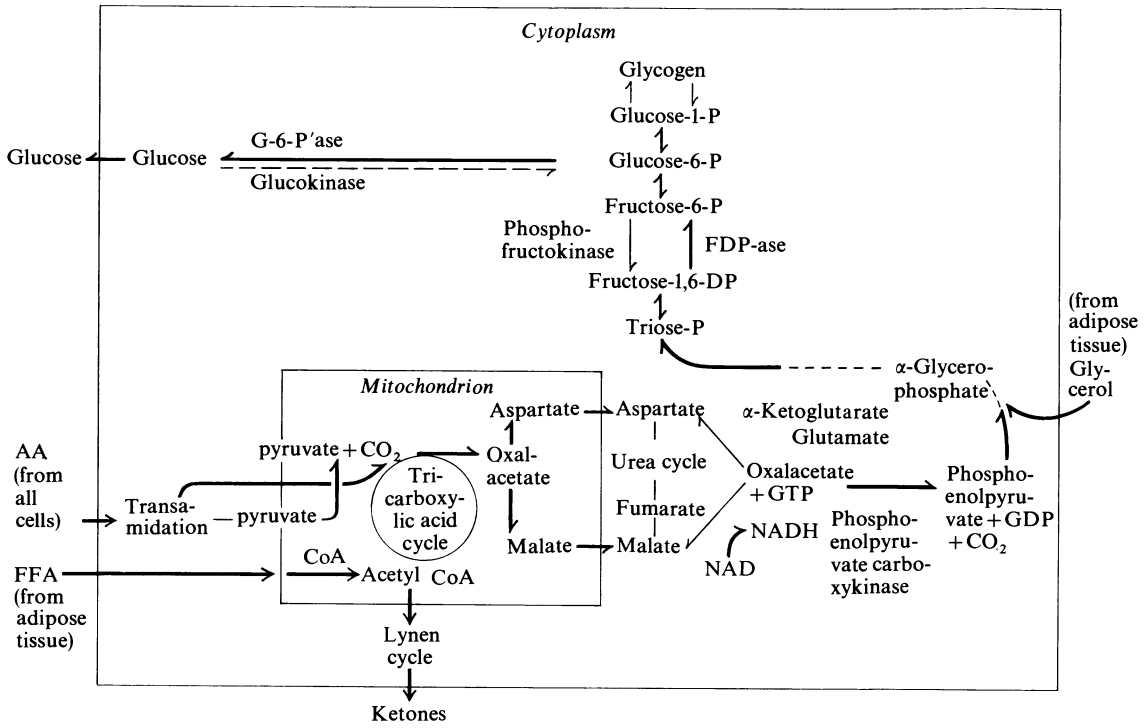


Fig. 3. Gluconeogenesis begins with the transamidation of amino acids to pyruvate and intermediary products of the tricarboxylic acid cycle. The flux of aminoacids from the periphery to the liver increases in anti-anabolic, catabolic situations such as fasting and diabetes and under the influence of glucocorticoids. As has been shown recently, free fatty acids appear to activate gluconeogenesis. Free fatty acids are converted to acetyl coenzyme A in the mitochondria and compete with pyruvate for coenzyme A which serves for its activation to acetyl-Co A by pyruvate oxidase. Acetyl-coenzyme A inhibits this enzyme. In this way, more pyruvate becomes available for conversion to oxalacetate. This latter substance can only be transferred to the cytoplasm after conversion to malate or fumarate and can then be used for gluconeogenesis after reconversion to oxalacetate. The mitochondrial conversion of oxalacetate to malate is accelerated by $NADH^+$ produced by the oxidation of free fatty acids. The activities of the key enzymes in gluconeogenesis (transaminases, phosphoenolcarboxykinase, pyruvate carboxylase, fructose-1,6-diphosphatase and glucose-6-phosphatase) are elevated when gluconeogenesis is stimulated by insulin deficiency, glucocorticoid excess or both. It has not been clarified whether this increase in enzyme activities is due to substrate induction or another type of enzyme induction. This scheme should, above all, stress the significance of the supply of free fatty acids to the liver for the activation of gluconeogenesis

ideal body weight. About 2/5 of the total calories are covered by carbohydrate. This percentage is fundamentally altered during fasting. The organism now uses up its fat reserves. The carbohydrate reserves consist of a maximum of 150 g each in the liver and muscle and are rapidly spent. Glucose consumption now contributes only about 10% to total calorie expenditure, the rest being covered by fat. Since the glycogen reserves are used up after approximately 48 hours, the liver must now supply glucose. The liver, the proximal renal tubules and the mucosa of the small intestine contain glucose-6-phosphatase, an enzyme which splits off glucose from glucose-6-phosphate and releases it into the blood.

The calorie requirement of the brain is about 400 calories per day, so that during fasting major portions of the glucose produced via gluconeogenesis are used up by the brain, whereas most other cells oxidize mainly free fatty acids and ketone bodies.

The acute release of glucose from the liver necessitates an activation of glycogenolysis. Adrenaline and glucagon increase glycogenolysis by activating phosphorylase. This activation occurs, as in muscle, through the so-called "second messenger", cyclic 3′,5′-adenosine monophosphate. Glucagon stimulates adenylcyclase and the 3′,5′-adenosine monophosphate formed in turn stimulates phosphorylase kinase which converts phosphorylase B to phosphorylase A (SUTHERLAND, 1965). This enzyme is now responsible for the splitting off of the marginal glucose residue of glycogen and its conversion to glucose-1-phosphate. According to recent investigations, it seems probable that the activation of phosphorylase is the major physiologic action of glucagon (SOKAL, 1966). Whereas very small physiologic doses of glucagon stimulate

glycogenolysis, larger doses of adrenaline are needed to achieve the same effect.

This acute regulation of blood sugar through glycogenolysis is supported by hepatic gluconeogenesis, which is responsible for the maintenance of normal blood glucose and glycogen stores during fasting. The main precursors of glucose formed via the gluconeogenetic pathway are certain amino acids and glycerol.

Amino acids are deaminated and transaminated to pyruvate or to intermediate products of the tricarboxylic acid cycle and can form glycogen and glucose. Glycerol liberated by lipolysis of triglycerides is the second most important endogenous precursor of glucose. At a caloric requirement of 2500 calories, about 2000 calories are generated by the combustion of fatty acids and ketones (210 g). Since these calories are liberated mainly from triglycerides, about 25 g of glycerol are released from adipose tissue at the same time and can then be converted to glucose. The remaining amount of glucose, approximately 75 g, arises from the amino acids, as has already been mentioned. The nitrogen of these amino acids appears in the urine (10 g of nitrogen correspond to 62 g of protein).

The regulation of gluconeogenesis by hormones and substrates is extremely complex. Glucagon first induces glycogenolysis and subsequently leads to increased gluconeogenesis (EXTON, 1966). A rise in the concentration of free fatty acids also leads to an increase in gluconeogenesis (STRUCK, 1965). The work of LARDY and his team has led to a better understanding of the intermediary metabolism of the liver and the most important controlling points (Fig. 3) (LARDY, 1965).

3. Chemistry, Synthesis, Biosynthesis, Secretion and Effects of Insulin

a) Chemistry and Synthesis

BANTING and BEST in Canada and PAOLESCO in Rumania succeeded in extracting insulin from the pancreas in 1922. This event precipitated tremendous research efforts to elucidate the chemical and biological properties of insulin. It led from its purification by ABEL (1926) to the clarification of the structural formula by SANGER (1955), to its synthesis (MEIERHOFER, 1963; KATSOYANNIS, 1964; ZAHN, 1967; Institute of Biochemistry, Academia Sinica, 1966).

b) Biosynthesis and Secretion of Insulin

STEINER (1967) has demonstrated the presence of a precursor of insulin, proinsulin, in the pancreas, from which insulin is split off by the catalytic action of several enzymes. Proinsulin is synthesized by the ribosomes and transferred to the Golgi apparatus where most of the splitting of proinsulin to insulin takes place. Insulin is then packed into granules which are lined up along microtubules close to the membrane of the B-cell. The biosynthesis of insulin

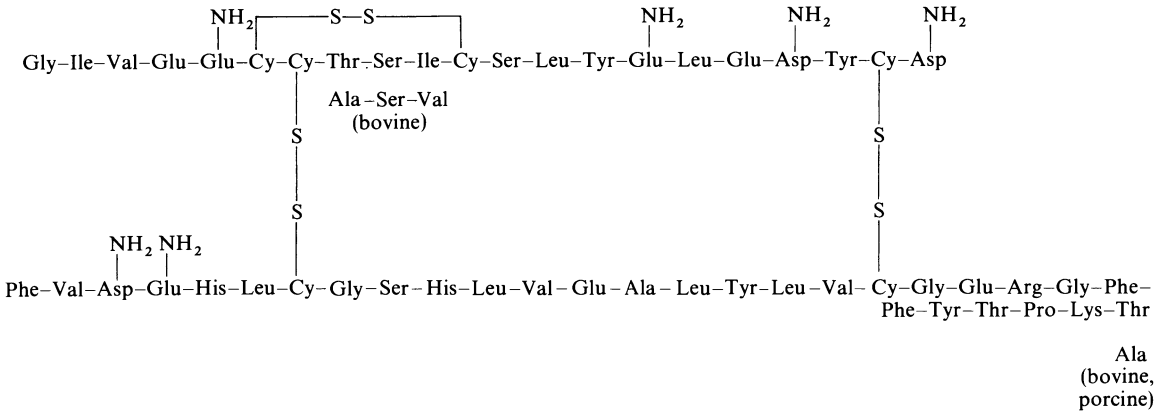


Fig. 4. Human insulin is a protein with a molecular weight of 5734. The isoelectric point lies at pH 5.6. It consists of 2 polypeptide chains, the A and the B chain which are linked by 2 disulfide bridges. The A chain with 21 amino acids contains another disulfide intra-chain linkage. The B chain is composed of a total of 30 amino acids. The biologically active centre of insulin is unknown. Small changes of the structure appear to alter the spatial arrangement of the insulin molecule so that biological activity is lost. Splitting of the disulfide bonds, alterations of the two histidine residues and splitting off of the 8 C terminal amino acids of the B chain result in loss of activity. The removal of the C-terminal threonine alone does not change the activity of insulin, this is changed when the end terminal asparagine of the A-chain is simultaneously removed (SLOBIN, 1963). The amino acids in positions 8–10 of the A chain differ in the human, bovine, procine and sheep insulins. A comparison between guinea-pig and human insulin shows that they differ in 14 amino-acid residues. The chemical synthesis of the A and B-chain by the conventional methods of peptide synthesis and by the solid-state method of MERRIFIELD (1967) no longer appears to present great difficulties. However, the linkage of the disulfide bonds and the combination of the two chains to form insulin has not yet been possible on a large scale

should not be confused with the process of insulin secretion. Different mechanisms are involved in insulin secretion: granules containing insulin are surrounded by the cell membrane and expelled by the process of emeiocytosis or exocytosis. Whether or not another secretory mechanism exists is not known (LACY, 1965). Nothing is known about the regulation of insulin biosynthesis. The islet cells seem to know when to produce new insulin, namely when insulin is being secreted and the insulin content of the cell decreases.

c) Regulation of the Secretion of Insulin

Until a few years ago, insulin secretion was believed to be controlled only by the blood-sugar level. In the meantime, a large number of substances which promote or inhibit insulin secretion has been discovered. Since CONARD's investigations on glucose assimilation (1955) it has been established that insulin secretion is stimulated more markedly by orally administered glucose than by intravenously injected glucose. SAMOLS (1965) and other authors (KETTERER, 1967) have repeatedly shown that glucagon stimulates the secretion of insulin even before the blood sugar rises. Following oral glucose, glucagon seems to be secreted before insulin secretion is increased (SAMOLS, 1965). This sequence of events tells us nothing about the importance of glucagon as an insulin secretagogue. Secretin given intravenously also causes insulin release (DUPRÉ, 1966). Some authors have a concept of a whole chain of gastrointestinal hormones (pancreozymin, gastrin, secretin, glucagon) which have to be secreted in the right sequence to lead to a timely insulin secretion (UNGER, 1967).

In addition to glucose, amino acids are also physiologic stimulators of insulin secretion. It has been known for a long time that leucine stimulates the secretion of insulin and that it can cause hypoglycemia (SCHWARTZ, 1959). A mixture of amino acids and single amino acids can also stimulate insulin secretion. In order of potency these are: arginine, lysine, leucine, phenylalanine, valine and methionine (FAJANS, 1967). Leucine is the only amino acid that causes hypoglycemia. In contrast to leucine, the action of the other amino acids is not potentiated but rather diminished by prior treatment with sulfonylureas. Leucine and all other amino acids seem to stimulate insulin secretion through different mechanisms. The effects of glucose and arginine on the B-cell are mutually potentiated. The insulinotropic action of amino acids is of phylogenetic interest. Certain fish and other animals live on protein and fat alone. In these animals, insulin appears to affect in particular the transport of amino acids into the cells and protein synthesis. It is reasonable to assume that insulin secretion is regulated mainly by the plasma concentration of amino acids in these animals.

Glucose is the main physiologic stimulus of insulin secretion in man. The significance of glucagon and secretin is not yet certain. It is possible that cyclic 3′,5′-AMP, the "second messenger", is also a mediator of insulin secretion (MALAISSE, 1967).

Adrenaline and noradrenaline prevent an adequate insulin release in response to glucose. Whether physiologic or pharmacologic concentrations of catecholamine are needed for inhibition of insulin secretion has not been assessed (PORTE, 1968). Hypokalemia can also lead to paralysis of the secretory process in the B-islet cells and the reduced glucose tolerance in patients with aldosteronism is related to hypokalemia (GRODSKY, 1966). Other ions, in particular Ca^{++} influence insulin secretion as well (MILNER, 1967; MALAISSE, 1971).

d) Pharmacology of Insulin Secretion

The diabetogenic effect of diazoxide (FAJANS, 1966; GRABER, 1966) is due mainly to inhibition of insulin secretion. Mannoheptulose (SIMON, 1966) is a very potent inhibitor of insulin secretion. Its effects are reversible, the side effects on liver metabolism are negligible. The original observation made by LOUBATIÈRES (1957), that sulfonylureas have a hypoglycemic effect by way of B-islet cell stimulation, has stood up to all criticism. Estimations of immunoreactive insulin in the blood before and after acute intravenous administration of sulfonylureas show that insulin secretion is stimulated within a few minutes (SAMOLS, 1965). Insulin secretion after sulfonylurea administration is short-lived under normal circumstances and prolonged in patients with B-islet cell adenoma.

Pentoses and pentitoles promote insulin secretion. It is assumed that these sugars are funneled into the pentose-phosphate shunt and that they supply extra energy for insulin secretion (KUZUYA, 1966; HIRATA, 1968). No other hypoglycemic drug acts via the B-islet cells (biguanides, synthalin, salicylic acid, hypoglycine).

e) Inactivation of Insulin in the Organism

It is occasionally stated that hormones are destroyed while acting on the receptor. This certainly does not apply to insulin. Intact adipose tissue, which is one of the main target

organs of insulin, inactivates hardly any insulin (PIAZZA, 1959).

Insulin has a very short biologic half-life. In the rat it is 3 min (BÜRGI, 1963), in the dog about 20 min (CAMPBELL, 1967), and in the human about 10 min (WELSH, 1956; CERASI, 1964). Insulin is split into A- and B-chains, mainly in the liver, by the enzyme insulin-glutathione-transhydrogenase (TOMIZAWA, 1962). This enzyme is also present in the kidneys, which contribute to the rapid inactivation of insulin. Small amounts of insulin are excreted in the bile (DANIEL, 1967).

Insulin is filtered through the glomerula and is almost completely reabsorbed. Nevertheless, about another 1% of the total amount of insulin secreted is excreted as such in the urine in conditions of tubular dysfunction (CHAMBERLAIN, 1967).

The biologic half-life of insulin is prolonged in people who have been treated with insulin over a long period of time (WELSH, 1956). Insulin antibodies then present in blood bind insulin so that enzymatic degradation by insulin-glutathione-transhydrogenase is prevented. Insulin has a prolonged action since it cannot be broken down because it is bound to the antibodies and is released only gradually (FANKHAUSER, 1963; CROUGHS, 1965).

f) Insulin in Blood

Insulin forms polymers in neutral solution. It is assumed but has not been proved that insulin circulates as a dimer or tetramer in blood. Until now, no specific carrier protein of insulin has been found in blood, although for some time the alpha-2-macroglobulins were considered to have this function (ZAHND, 1963). Endogenous human insulin reacts with insulin-antibodies which were produced in the guinea pig by injecting crystalline pancreatic insulin.

One cannot conclude from this that crystalline insulin extracted from the pancreas and filled into ampoules is the same chemical substance as the endogenous insulin circulating in the blood, although the antibodies cannot differentiate between the two.

Insulin in blood can also be assayed biologically *in vitro* using rat adipose tissue. The immunoreactive insulin in the serum corresponds to the insulin-like activity (ILA) which is inhibited by antibodies (FROESCH, 1967). The normal values of ILA for normal subjects in the fasting state lie between 10 and 30 micro-units per ml of serum, the average value being 20 micro-units per ml. The immunoreactive insulin increases rapidly after a single intravenous injection of glucose, reaches a maximum of 100 micro-units per ml after 2–5 min and falls to the original value 30–60 min after glucose administration (SAMOLS, 1965). The concentration of insulin in blood and its rise after glucose administration appear to be dependent on body weight and age, among other factors. Overweight patients with normal glucose tolerance often show an excessive rise in plasma insulin in response to glucose administration (KARAM, 1963).

g) Biological "Insulin-Like" Substances in the Serum

The serum contains protein which promote the uptake of glucose by muscle and adipose tissue *in vitro*, and which also simulate the antilipolytic effect of insulin. The so-called "nonsuppressible" or "atypical" insulin activity of the serum is the most important of these substances (FROESCH, 1967). Nonsuppressible ILA is not inhibited and is not bound by antibodies to insulin. Serum contains about 200 micro-units of nonsuppressible ILA and 20 micro-units of suppressible ILA. Suppressible ILA corresponds to immunoreactive plasma insulin. The nonsuppressible ILA can probably not be attributed to a single molecule. The main part is a protein with a molecular weight between 70000 and 150000 (BÜRGI, 1966; JAKOB, 1968). This molecule appears to be inactive *in vivo*, at least under standard conditions. The reason for this is not clear. Nonsuppressible ILA is not decreased in diabetics, in diabetic ketosis, or in the pancreatectomized dog. These and other observations make it seem unlikely that nonsuppressible ILA has any physiologic significance in glucose homeostasis or that it originates in the pancreas (FROESCH, 1967). The hypothesis according to which the large molecule with nonsuppressible ILA might pass through the cell membrane only when capillary permeability is increased (muscular activity, inflammation) is still open to discussion. In this way, in contrast to insulin, whose action is systemic, nonsuppressible ILA might act locally where extra glucose is needed.

A small protein with a molecular weight of 8000 with nonsuppressible ILA has been extracted from serum and partially purified (BÜRGI, 1966). It has the same effects as insulin on adipose tissue, and appears to stimulate glucose uptake by muscle more actively than its uptake by adipose tissue (FROESCH, 1966). The purified molecule with nonsuppressible ILA contains neither A- nor B-chain and probably has 3 disulfide bonds. It is an interesting molecule. The elucidation of its structure may help to find the biologically active centre of insulin. Lately, nonsuppressible ILA has been

shown to be a potentgrowth factor for fibroblasts in culture and a potent sulfation factor (somatomedin) (MORELL, 1973; ZINGG, 1973).

h) The Action of Insulin on the Transport of Glucose

Today, there is absolutely no doubt that the theory first formulated by LEVINE and GOLDSTEIN (1955), according to which insulin accelerates glucose transport into the cell, is correct. This effect of insulin on glucose transport has been investigated thoroughly in muscle and in adipose tissue (PARK, 1961; CROFFORD, 1965; MORGAN, 1964). The action of insulin on glucose transport sets in as soon as insulin comes into contact with the cell, and stops immediately when insulin is neutralized by anti-insulin serum (FROESCH, 1963). It is to be assumed that insulin combines in a reversible manner with the membrane and that it changes its configuration. Insulin accelerates the transport of glucose in adipose tissue *in vitro* even in concentrations as low as 1–10 micro-units per ml. These concentrations correspond to those found in the serum of fasting men. When glucose transport in adipose tissue and in muscle is accelerated maximally by insulin *in vitro*, glucose phosphorylation in the cell may become a rate-limiting factor for total turnover, and glucose in the free form may accumulate in the cell (FROESCH, 1963; MORGAN, 1961). It is not likely that this ever occurs under physiologic conditions. Insulin is released in particular after the intake of starch or glucose, which accounts for the fact that glucose reaching the blood during the resting state is stored in the tissues. Glucose is not oxidized, but rather stored. The regulation of glucose uptake by insulin is different from stimulation by muscular exercise. Whereas a glucose load can be stored only under the influence of insulin, increased energy requirement of the cell determines the glucose uptake in conditions of exercise and glucose need.

i) Effects of Insulin which are Independent of Glucose (see also Fig. 5)

The transport theory is correct, but the effect on glucose transport is certainly not the only action of insulin. Insulin promotes the transport of amino acids into muscles and fat cells and their incorporation into protein (MANCHESTER, 1965; SCHARFF, 1965; CARRUTHERS, 1962; KRAHL, 1964). The uptake of potassium by the cell is only increased in the presence of glucose. Insulin raises the resting electric potential of the adipose-tissue cell in the absence of glucose in the medium (BEIGELMAN, 1962). The anti-lipolytic effect of insulin is of special importance to consideration of the metabolic disorders in diabetes mellitus. Insulin inhibits the release of glycerol and free fatty acids by adipose tissue of the rat regardless of whether glucose is present in the medium or not (FROESCH, 1965; JUNGAS, 1963; MAHLER, 1964). Triglyceride-lipase determines the rate of lipolysis and is inhibited by insulin. In addition, insulin appears to influence glycogen metabolism. Insulin inhibits phosphorylase and activates glycogen synthetase in such a way that glycogen is less rapidly broken down and more rapidly synthesized (JUNGAS, 1966; FROESCH, 1967). It

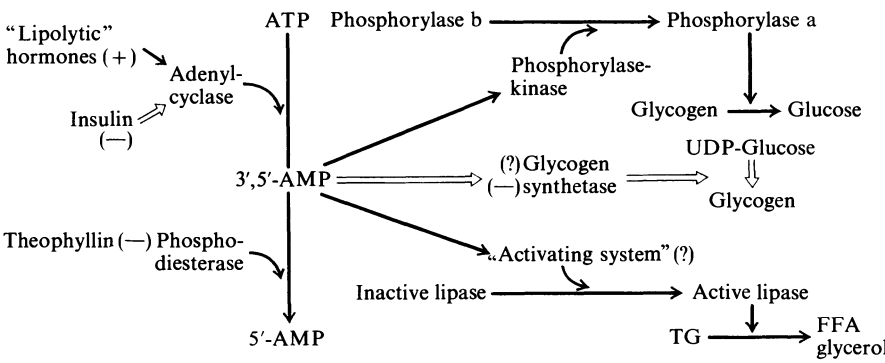


Fig. 5. The action of insulin on lipolysis and glucose metabolism is partly independent of glucose in the medium and occurs as rapidly as its effect on glucose transport. The concentration of cyclic 3',5'-AMP in adipose tissue decreases under the influence of insulin and increases under stimulation of lipolysis by glucagon, the adrenal medullary hormones and ACTH. These hormones seem to influence the formation of 3',5'-AMP by activation of adenylcyclase. This enzyme is localized in the membrane and releases 3',5'-AMP, the so-called "second messenger", into the cell. There it activates lipase and phosphorylase and probably inhibits glycogen synthetase. The causal relations between 3',5'-AMP and the activation of enzymes is, however, not yet fully explained. The effect of 3',5'-AMP is much less pronounced or absent in the homogenate or in the case of purified enzymes, and it will be difficult to prove that activation of these enzymes is really caused by an increase in the concentration of 3',5'-AMP and not simply by a concomittant phenomenon.

is interesting to note that all three enzymes involved are probably bound to their insoluble substrate in the cell. This assumption has led to speculations that insulin which does not penetrate into the cell itself may liberate some substance from the membrane into the cell, which could then influence the activity of these structurally bound enzymes within the cell. BUTCHER (1966) has demonstrated that insulin inhibits adenylcyclase which converts ATP to cyclic 3′,5′-AMP, the so-called second messenger. This cyclic compound promotes all processes in adipose tissue which provide energy for other tissues, i.e. lipase and phosphorylase. Inhibition of the formation of cyclic 3′,5′-AMP would, in contrast, promote the storage of glycogen and fat. Thus, in addition to its stimulation of glycogen and lipid storage through increased glucose uptake, insulin also inhibits the intracellular breakdown of glycogen and fat. These interrelations are presented in Fig. 5.

k) Effects of Insulin on Protein Synthesis (see also Fig. 6)

One characteristic of the diabetic state is a partial loss of activity of certain enzymes in various tissues. Hexokinase and glucokinase activities are decreased (SOLS, 1965). In adipose tissue of diabetic animals the activity of lipoprotein lipase is low, while in muscle it is higher than normal (HOLLENBERG, 1965). Insulin administration for 12 to 24 hours will normalize these enzyme disorders in diabetic tissue. It is not yet certain whether insulin restores protein and enzyme synthesis to normal by acting

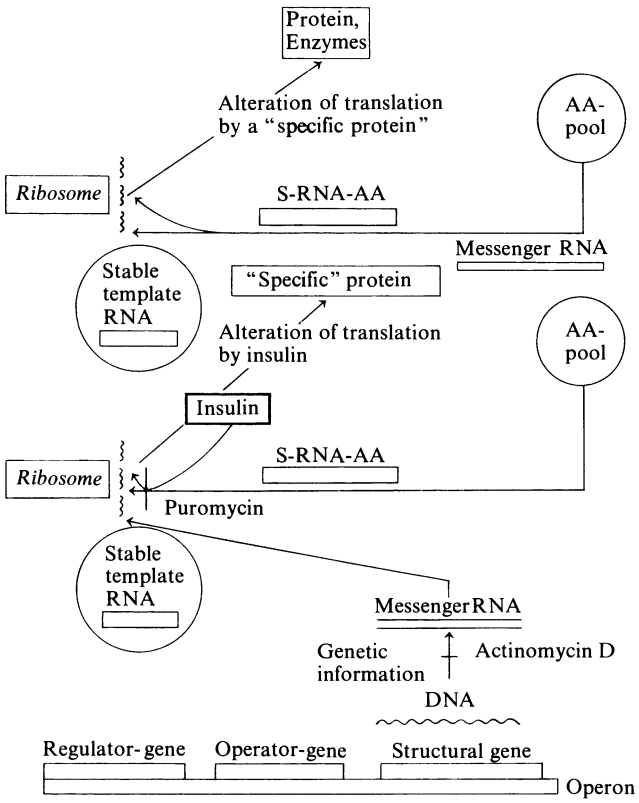


Fig. 6. Effects of insulin on protein synthesis. With few exceptions insulin acts only on intact cells. It stimulates the synthesis of enzymes which are important for the storage of glycogen and fat (among others: glucokinase, glycogen synthetase, glucose-6-phosphate dehydrogenase, pyruvate kinase, citrate cleavage enzyme, acetate thiokinase) and decreases the synthesis of those enzymes in the liver which determine the rate of gluconeogenesis (glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenolpyruvate-carboxy kinase, pyruvate carboxylase). It has not been possible to demonstrate that insulin acts directly on enzyme synthesis. Some insulin effects are certainly secondary to its primary action on the cell membrane (glucose transport, adenylcyclase). The work of WOOL is very important since he has demonstrated for the first time a clearcut action of insulin on the ribosomes. According to WOOL (1965, 1967) insulin alters the translation of genetic information at the level of the ribosomes. Since this effect is inhibited by puromycin and cycloheximid but not by actinomycin D, insulin does not appear to affect transcription, i.e. the transmission of genetic information from DNA to RNA but rather protein synthesis at the level of the ribosomes. According to WOOL insulin brings about the synthesis of some "specific" protein which then induces a chain of further reactions resulting in the synthesis of particular proteins and enzymes. This interpretation of the action of insulin on ribosomes still is somewhat speculative. (According to WOOL, 1966)

directly on DNA or RNA synthesis or by way of its effects on the cell membrane and intermediary metabolism. WOOL's work is very interesting and important. He was the first to show that insulin acts on cellular components, the ribosomes. According to WOOL (1965, 1967), insulin changes the translation of the genetic information into protein synthesis at the ribosomes in a short time. Since actinomycin D does not influence this effect of insulin, whereas puromycin and cycloheximide do, insulin does not seem to act on transcription, i.e. the transmittance of the genetic information from DNA to RNA, but rather on protein synthesis at the ribosomes. According to WOOL, insulin stimulates the synthesis of a "specific" protein, which could further influence the synthesis of other protein molecules and enzymes in a chain of reactions. This interpretation of the action of insulin on isolated ribosomes is, however, still a matter of speculation and debate.

l) The Action of Insulin on the Liver

The question of whether insulin influences liver metabolism directly appeared to be solved when no action was detected on liver slices *in vitro* (RENOLD, 1955). Insulin does not act on glucose transport into the liver cell. The concentration of glucose in the cytoplasm of the hepatic cells is roughly the same as that in the blood in the presence and absence of insulin (CAHILL, 1958). However, in recent times, different research groups have demonstrated unequivocally that insulin decreases the release of glucose, the rate of gluconeogenesis and the production of urea both *in vivo* and in the perfused liver *in vitro* (COMES, 1961; DE BODO, 1963; MADISON, 1965). The mechanism of this hepatic action of insulin is not explained. Since some of these insulin effects are inhibited by substances which block protein synthesis, it may be assumed that insulin promotes the synthesis and formation of enzymes and exerts some of its effects on the liver in this way (SOLS, 1965). Insulin also inhibits the biosynthesis of enzymes essential to gluconeogenesis (WEBER, 1965).

4. The Acute Metabolic Disorder in Diabetes and Diabetic Coma

E. R. FROESCH and P. H. ROSSIER

a) Consequences of Absolute Insulin Deficiency (see Fig. 7)

The acute metabolic disturbance in diabetes mellitus is the direct result of the complete or almost complete deficiency of insulin. Diabetic coma in man can be exactly reproduced in experimental animals by destruction of the B-islet cells with alloxan or streptozotocin, by total pancreatectomy, by the inhibition of insulin secretion with mannoheptulose and by blockade

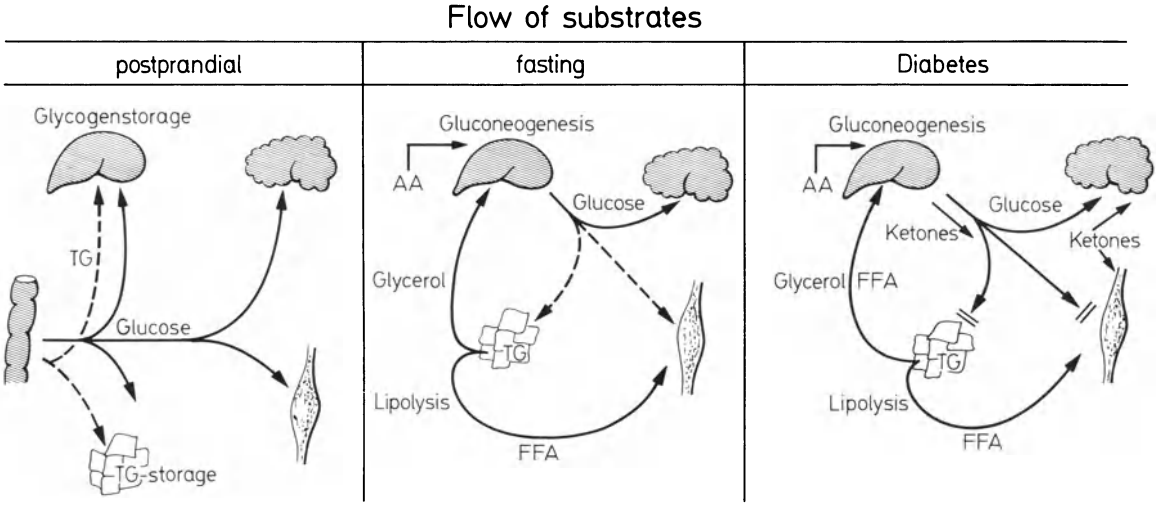


Fig. 7. Scheme of the metabolism of the most important fuels after eating, fasting and in the acute diabetic stage. Insulin is an absolute prerequisite for the storage of glucose as glycogen and fat and for the uptake and storage of fat in adipose tissue. Insulin increases glucose uptake of adipose tissue and increases the activity of lipoprotein lipase which enables the fat cell to hydrolyze chylomicrones and to take up the fatty acids which are esterified with α-glycerophosphate and stored as triglycerides. Free fatty acids released by adipose tissue are the major fuel of the fasting and diabetic organism. The massively increased release of free fatty acids by diabetic adipose tissue is due to decreased reesterification and stimulated lipolysis. Part of the free fatty acids taken up by the liver are oxydized to ketone bodies which also serve as a fuel but eventually flood the diabetic organism. (From FROESCH *et al.*, 1966)

of circulating insulin with anti-insulin serum. The animals die in diabetic ketosis 48 hours after the beginning of an infusion with high doses of neutralizing anti-insulin serum (ANDERSON, 1963). Experimental hyperglycemia and ketosis are reversible when insulin is administered at the right time and in sufficiently high doses.

Hyperglycemia arising within a few minutes after withdrawal of insulin is due to the immediate cessation of glucose transport into muscle and adipose tissue cells. The half-life of glucose increases rapidly in the rat from the normal 20 min to between 40 and 60 min after the injection of anti-insulin serum. The liver contributes to the hyperglycemia only after a while by increasing gluconeogenesis.

Under such conditions, glucose is no longer a major substrate for cellular metabolism. Free fatty acids and ketones serve as the main substrate. They arise entirely from adipose tissue. Lipases liberate free fatty acids from depot triglycerides. They are in part reesterified. The release of free fatty acids from adipose tissue is determined by the intracellular reesterification with alphaglycerophosphate derived from glucose and by the activity of the lipases (VAUGHAN, 1965). When insulin is absent, no more glucose is available for the reesterification of fatty acids, so that they are released into the blood. It is still unknown to what extent direct inhibition of lipolysis of adipose tissue by insulin and the removal of this block determine the release of free fatty acids. Stimulation of lipolysis by lipolytic hormones and inhibition of lipolysis by insulin fulfill all criteria of a neatly regulated metabolic system. Lipolysis is not only active when insulin is absent and lipolytic hormones, such as adrenaline, circulate in increased amounts. Lipolysis is also very active when animals are fed after prolonged periods of fasting, and lipolysis is then especially strongly inhibited by insulin (FROESCH, 1965).

During insulin deficiency the organism switches over from the oxidation of glucose to the consumption of fat, i.e. free fatty acids. Adipose tissue is the only organ which possesses an unlimited capacity for the storage of potential energy in the form of neutral fats. During fasting and in the diabetic state it provides almost all the energy for all other tissues. Only the brain and the erythrocytes continue to use glucose.

The release of free fatty acids from adipose tissue during fasting slightly exceeds the requirements of the organism. During complete insulin deficiency the free fatty acid release is no longer adjusted to the actual needs and ketone bodies are formed from free fatty acids.

b) Formation of Ketone Bodies and Ketosis

Ketone bodies are formed exclusively in the liver from acetyl CoA in a cycle described by LYNEN (1958). Acetyl-CoA accumulates in the liver by excessive beta-oxidation of fatty acids. During glycolysis, every molecule of pyrurate may give rise to a molecule of oxalacetate from which citrate is derived for the Krebs cycle. The same does not apply to the breakdown of fatty acids. Acetyl-CoA increases and ketone bodies are formed. This is in no way a process limited to diabetes mellitus. Whenever the liver is flooded with free fatty acids formation of ketone bodies is increased. Even the non-diabetic liver extracts a large percentage of the fatty acids supply and oxidizes them to ketone bodies. The formation of ketone bodies is for the most part a function of the concentration of free fatty acids in the blood (AYDIN, 1963).

Depending on the pH of the urine, the ketoacids are excreted partly as undissociated free acids and partly as sodium and potassium salts. During ketosis, sodium and potassium is lost. Since the water loss is greater than the cation loss, hypokalemia becomes apparent only during rehydration.

c) Causes of Hyperosmolarity and of Dehydration (see Fig. 8)

Insulin deficiency is the cause of the water and electrolyte inbalance in diabetic coma. These processes are presented schematically in Fig. 8. The first phase of diabetic decompensation begins with a rise in blood sugar. Serum osmolarity rises and for compensation water flows from the cells into the extracellular space. Temporary hypervolemia results and leads at first to a brief fall in aldosterone secretion and renal loss of sodium and chloride. On the other hand, the osmoreceptors in the hypothalamus are stimulated to release ADH so that more water is reabsorbed in the kidneys. This regulation mechanism may function only in the first phase of diabetic decompensation and later be overrun by a much more important mechanism. The renal tubules can reabsorb a maximum of 350 mg of glucose per minute. When more glucose is filtered through the glomerula it is lost in the urine. On the other hand, the concentrating capacity of the kidneys is limited to a maximum of 1000 mOsmol/l. With a blood sugar of 712 mg% and a glomerular filtration rate of 125 ml per minute, 540 mg of glucose or 3 mOsmoles are excreted per minute. The kidney needs at least 3 ml of water per minute or 180 ml per hour for the excretion of this amount of glucose alone. In addition, the concentrating

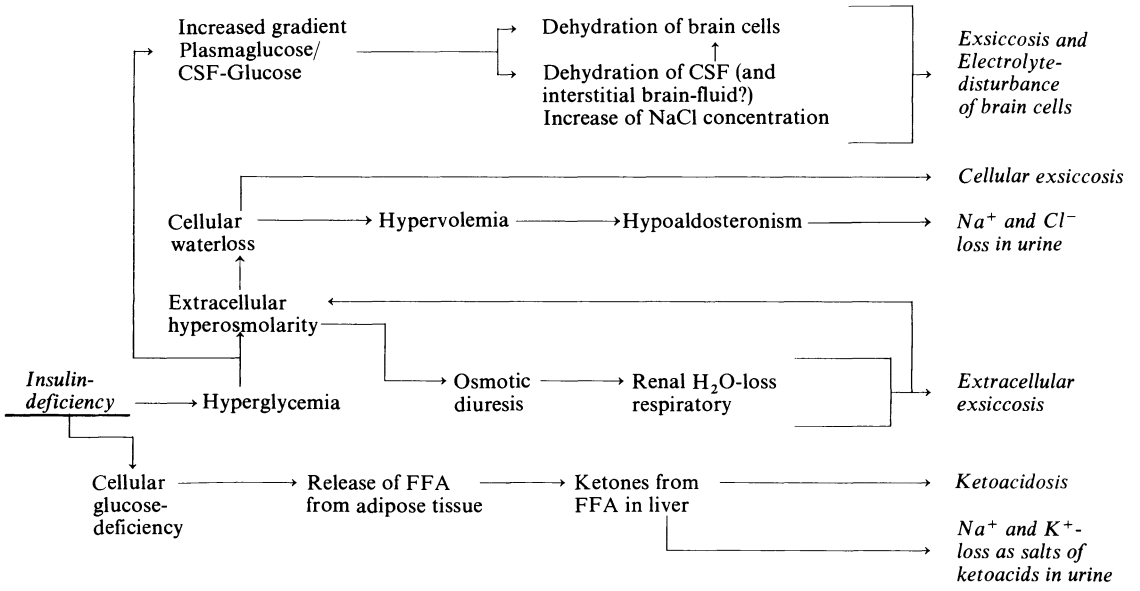


Fig. 8. Causes of hyperosmolality, exsiccosis and ketoacidosis in diabetic decompensation. (From FROESCH *et al.*, 1966)

capacity of the kidneys is limited during osmotic diuresis and seldom exceeds 600–800 mOsmoles/l. As soon as this enormous loss of water is no longer compensated for by drinking, a water deficit develops leading to severe hypertonic dehydration. This renal water deficit is further increased by respiratory loss of water due to the deep Kussmaul-type breathing.

The hyperosmolarity of the serum, primarily due to hyperglycemia and water loss, now increases further since the end-products of cellular metabolism can no longer be excreted because of renal failure due to circulatory factors. End-products of metabolism such as urea accumulate. As mentioned above, the organism attempts to correct hyperosmolarity of the serum by the flow of water from the cells into the interstitial space and into the blood. With the exception of erythrocytes and liver cells, most other cells in the body contain no free glucose. The intracellular osmolality of these cells is not affected directly by the rise in blood sugar, but secondarily by the cellular loss of water into the interstitial fluid and the blood.

d) Effects of Ketoacidosis, Hyperosmolarity and Dehydration on the Metabolism of the Brain

What are the causes of the disturbance of brain metabolism leading to cerebral coma? This question cannot yet be adequately answered even now. There is a pronounced potassium deficit in diabetic ketosis. Intracellular potassium is replaced by sodium and hydrogen ions. We would, therefore, expect an intracellular acidosis also in diabetic coma. A massive increase of CO_2 causes narcosis. CO_2 content and pressure in the plasma are especially low in metabolic acidosis due to hyperventilation. Is the metabolic disturbance of the brain directly responsible for the coma? Is the cellular dehydration responsible? During hyperglycemia, the sugar level in the cerebrospinal fluid is much lower than the blood sugar, so that an osmotic gradient is built up between the blood and the cerebrospinal fluid. It is temporarily compensated by a loss of water from the cerebrospinal fluid and a resultant rise of the sodium chloride concentration (FROESCH, 1967). The ratio of blood glucose to cerebrospinal fluid glucose averages 1.9. The quotient is smaller at low blood sugar concentrations and rises to above 2 at blood sugar values above 1000 mg%. A comparison between the chemical composition of serum and that of cerebrospinal fluid (CSF) shows that CSF sodium is always more or less markedly elevated in diabetic coma, in spite of normal sodium levels in the serum. Can we conclude from this that the brain cells are bathed in a fluid in which the sodium content is elevated and rises as the glucose gradient between blood and CSF rises? Whereas the cerebrospinal fluid is formed by the ependyma of the choroidal plexus and also partly reabsorbed there, we know little about the interstitial fluid of the brain and its formation. The interstitial space which is thought to make up to 30% of the weight of the brain cannot be

localized under the electron microscope (TSCHIRGI, 1960). The capillaries are densely surrounded by the foot-like elongations of the astrocytes. A few authors hold that the so-called blood CSF barrier is formed by these parts of the astrocytes and the astrocytes themselves. The formation of the CSF is certainly not the same as the formation of the interstitial fluid of the brain. Furthermore, the CSF is renewed 2 to 4 times daily, a turnover which could not provide the brain with enough glucose and oxygen. What do we know about the blood/brain barrier in relation to glucose? According to TSCHIRGI (1960) the glucose content of the brain is, on average, 25–40% lower than the blood sugar and it changes in a parallel manner with the blood sugar. When the blood sugar rises to 800 and 1600 mg%, however, the glucose content of the interstitial fluid of the brain does not increase above 300 mg%. As a result of this, the ratios differ from those in the CSF, but they do indicate similar trends, i.e. an ever-increasing osmolality gap resulting in dehydration of the brain cells as the blood sugar increases. As early as 1932, ROSSIER made the observation that hyperventilation lasts longer than the acidosis of the blood and may occasionally lead to respiratory alkalosis. An explanation for this fact was found in recent investigations which show that the pH of the CSF lags behind the pH of the blood during treatment, since the organic acids pass the blood/CSF barrier only sluggishly. The low pH of the CSF continues to stimulate the respiratory center, even when the blood pH is already normal (see Table 1).

5. Diagnosis, Differential Diagnosis and Prognosis of Diabetic Coma

E. R. FROESCH and P. H. ROSSIER

a) Diagnosis and Differential Diagnosis

The diagnosis of a typical case of diabetic coma is very simple. In the majority of patients, diabetes mellitus was known to be previously present. We then have to differentiate between a hyper- and a hypoglycemic coma. The red, dry skin, the slow smoothing out of the elevated skin folds, the soft eye bulbs, the flat, fast pulse rate, the Kussmaul breathing, and finally the smell of acetone are unequivocal signs of the classical diabetic coma. Apart from the positive urine sugar and acetonuria, the determination of a highly elevated blood sugar is essential for the diagnosis. Today, this can be done rapidly with the Dextrostix at the bedside. Occasionally ketone bodies are also elevated when the blood sugar is very low during a prolonged hypoglycemic coma. It may then be mistaken for a diabetic coma since glucose may still be present in the urine from previous hyperglycemia. This stresses the importance of a rapid blood sugar determination for the diagnosis of diabetic coma.

Hyperosmolar, non keto-acidotic diabetic coma may present special difficulties (ROSSIER, 1961; STÖTTER, 1962). Often it is the first manifestation of diabetes mellitus, and the coma is not always preceded by a period of polyuria and polydipsia, especially when the renal function is already impaired in elderly patients. In addition, Kussmaul breathing and the smell of acetone are

Table 1. Blood and cerebrospinal fluid (CSF) in diabetic coma before, 6 and 24 hours after the beginning of treatment

	Predominantly ketoacidotic coma					
	Before therapy		6 hours after start of therapy		24 hours after start of therapy	
	Blood ($n=8$)	CSF ($n=8$)	Blood ($n=7$)	CSF ($n=7$)	Blood ($n=4$)	CSF ($n=4$)
Na^+ (meq/l)	135.8	156.0	138.8	147.0	140.5	151.0
Cl^- (meq/l)	95.8	134.7	101.1	123.5	96.0	116.7
Glucose (mg%)	845	518	337	377	318	226
Osmolality (mosmoles kg)	360	339	332	310	321	315
pH	7.05	7.10	7.35	7.20	7.42	7.31
Remaining acid (meq/l) (calculated)	30.4	17.0	15.8	15.6	11.5	13.8
	Predominantly hyperosmolar coma					
	Blood ($n=5$)	CSF ($n=5$)	Blood ($n=4$)	CSF ($n=4$)		
Na^+ (meq/l)	137.2	170.8	149.7	160.6		
Cl^- (meq/l)	97.0	150.6	101.7	126.0		
Glucose (mg%)	1764	802	734	715		
Osmolality (mosmoles kg)	453	441	418	416		
pH	7.19	7.23	7.37	7.28		
Remaining acid (meq l) (calculated)	21.4	8.0	18.8	19.4		

absent. The blood pressure often falls even in the early stages of hyperosmolar coma.

b) Prognosis of the Diabetic Coma

Diabetic coma carries an average mortality of 10–20%. The definition of diabetic coma is of extreme importance in a comparison of the results of different medical centers. This is brought out by the Frankfurt study (PFEIFFER, 1960). In this study 83 cases of diabetic coma were admitted from 1954 to 1959, and 16 of these died from the coma itself and a further 16 from other causes. Not one of the 215 diabetics referred in the same period for admission in the precomatose state died. These figures indicate how very important it is to start treatment as early as possible, since the prognosis gets rapidly worse with the duration of the unconsciousness. Of the 16 patients mentioned above, who died in and of the diabetic coma, the unconsciousness lasted longer than 10 hours before treatment was started in 14 patients, and was even longer in 2 patients in whom diabetes mellitus had not been diagnosed before the comatose episode. The prognosis is unfavorable in elderly patients, mainly because of the higher risk of cardiovascular complications. The longer diabetes has been present, the worse is the prognosis. In addition, all severe precipitating and concomitant illnesses, such as myocardial infarction, arteriosclerosis, gangrene, uremia and infections, influence the prognosis unfavorably. In contrast, the level of the blood sugar does not seem to be of importance for the prognosis. Several unfavorable prognostic factors not infrequently occur together in hyperosmolar diabetic coma. This is often seen in elderly patients with preexisting cardiovascular disease. In many, circulatory failure which may be resistant to therapy is present from the very beginning or may develop during the course of treatment.

6. Treatment of Diabetic Coma

a) Treatment Schedule in Use since 1960 in the Department of Medicine, University of Zürich (ROSSIER, 1960)

1. *Insulin by slow infusion:* Decrease of blood sugar, osmolality, free fatty acids, ketone bodies and amelioration of acidosis.
2. *Hypotonic fluid administration:* Correction of the exsiccosis and of the hypertonic dehydration.
3. *Na^+ administration, half of it being given as bicarbonate:* Compensation of the sodium loss and of the metabolic acidosis.
4. *K^+ administration starting between the 4th and 6th hours:* Compensation of the previous potassium loss and the potassium displacement into the cells during insulin therapy.

Intravenous fluid for diabetic coma:

1/3 as physiologic NaCl solution
1/3 as physiologic Na-bicarbonate solution (1/6 molar, 14 g/l),
1/3 as H_2O.

In uncomplicated coma:

1st hour	1 liter with 100 units of soluble insulin,
2nd hour to 4th	1 liter with 100 units of soluble insulin,
5th to 12th hours	1 to 2 liters with 50 to 200 units of soluble insulin,
13th to 24th hours	1 to 2 liters with insulin according to needs.

Potassium replacement: 20 to 40 meq/h (1.5–3 g of KCl).

Glucose: The hypotonic "coma" solution is replaced by a 5% glucose infusion when the blood sugar approaches 250 mg%.

b) Amount of Fluid (see Table 2)

Table 2. Water- and electrolyte deficit in diabetic coma according to various authors

	JOSLIN (1959)	MACH (1953)	DEROT (1961)	BUTLER (1950)
Water (ml)	6866	4000–5000	5000–8500	7000
Na^+(meq/l)	351	350– 700	350– 700	350
K^+ (meq/l)	493	400– 600	350– 420	420
Cl^-(meq/l)	430	250– 600	350	280

As already discussed in the section on the causes of dehydration, the water deficit exceeds the sodium deficit, resulting in hypertonic dehydration. Correspondingly, a hypotonic solution should be used for rehydration. It has been shown that the results of therapy obtained with hypotonic solutions are almost the same as those obtained with isotonic fluids. On the other hand, hypertonic solutions are strictly contraindicated.

The fluid deficit in diabetic coma varies from about 4 to 9 liters (average 5.5 liter), and this amount of fluid must be replaced. The rate of fluid administration must be adjusted to various factors. In the young diabetic without cardiac disease, 6 to 8 liters may be given intravenously in 24 hours. In the elderly patient fluid must be administered with caution. The danger of overloading the circulation and thus causing pulmonary edema is serious. Such patients should generally not be given more than 4 liters intra-

venously in 24 hours. As soon as these patients regain consciousness and as soon as the danger of aspiration is overcome, further rehydration can occur without any risk by the oral route.

c) The Composition of the Intravenous Solution

An essential requirement for any solution is its readiness for use at any time, so that treatment can be started promptly. For this reason JOSLIN (1959) recommended physiologic saline, although this solution is isotonic and not hypotonic. The major advantage of saline is that it is always ready and available.

The solution of 1/3 physiological saline, 1/3 physiological sodium bicarbonate and 1/3 water has the same advantage in being indefinitely stable (ROSSIER, 1960). This solution is hypotonic with an osmolality of 210 mosmol/l. The ratio of sodium to chloride is 2 : 1 and the bicarbonate content is 55 meq/l. The Hausmann solution for diabetic coma, commercially available in Switzerland, has a similar composition. Sodium lactate, which was formerly often used instead of sodium bicarbonate, has lost its place in the therapy of the diabetic coma, since spontaneous hyperlactatemia is relatively frequent in these patients (HUCKABEE, 1961). The organism should not be flooded with organic anions, the utilization of which is not guaranteed (SCHWARTZ, 1962).

Tris buffer has repeatedly been recommended for the treatment of coma. We have no personal experience of this use of the solution and do not understand the reasoning behind this therapy.

d) Treatment with Intravenous Insulin
(See Therapeutic Schedule)

Treatment with insulin, like fluid administration, must be started at once after the diagnosis of diabetic coma has been made. The extremely high doses of insulin often used are not necessary if insulin is administered in an intravenous drip. The biological half-life of insulin in the human is about 10 min. If 200 units of insulin are injected intravenously, 100 units are destroyed by the liver after 10 min, and only 12 units are circulating in the blood after 1 hour. The major part of the insulin is removed by the liver and inactivated before having exerted any effect on peripheral tissues. The situation is quite different in diabetics with extremely high titers of insulin antibodies in the blood which cause insulin resistance. The inactivated insulin bound to insulin antibodies circulates for much longer in the blood than free insulin. In such patients the circulating antibodies must first be saturated before insulin can act (FRANKHAUSER, 1964). In this case, single injections of high doses of insulin are indicated for the saturation of antibodies.

Subcutaneous or intramuscular administration of insulin achieves an insulin depot. The unknown factor in this type of insulin therapy is the absorption rate, which can be appreciably slowed down in diabetic coma because of the poor circulatory state. Therefore, we never know exactly how much insulin has already been inactivated, how much is still circulating in the blood and how much remains to be absorbed from the depot at any given time during treatment with subcutaneous insulin. The logical and correct treatment with insulin in an intravenous drip was not used previously because nothing was known about the stability of insulin in neutral or slightly alkaline solutions. VÖLLM (1960) tackled the problem of the stability of insulin in our laboratory and demonstrated that insulin lost none of its activity when it was stored at 37 °C for over 24 hours in a solution of physiological sodium bicarbonate. Since then we have not observed any failures to respond to an intravenous insulin drip. The favorable results previously reported have been fully confirmed in other hospitals and the intravenous insulin application is now routine in most Swiss hospitals. The dosage of insulin can be adjusted much more simply in this way than with subcutaneous injections. There is also no danger of hypoglycemia, since no depot of insulin is applied and since the action of the intravenously administered insulin lasts at the most 30 min to one hour after the drip is finished. We are convinced of the simplicity and of the practical advantages of the treatment with insulin in an intravenous drip, yet we do not know whether the prognosis of diabetic coma has been improved by this method. Despite rapid normalization of the metabolic situation, refractory irreversible circulatory collapse of unknown etiology still proves fatal to too many patients. The fatal outcome of the diabetic coma is frequently due to irreversible brain damage. However, not all cases can be explained in this way, since circulatory failure is not always preceded by severely disturbed brain function and deep coma.

e) The Dosage of Insulin

The principle that too much insulin at the beginning of the treatment of the diabetic coma can only help and causes no damage, is certainly correct. In our experience 100 units of insulin during the first hour after the onset of therapy, and a further 100 units in the following 3 hours given intravenously in a drip, exert optimal effects on the metabolism which can hardly be

surpassed by higher doses of insulin. Diabetics who have high antibody titers and are resistant to insulin are probably the exception to this rule and require much more insulin. ROOT recommended (1959) that the insulin dosage be chosen according to the blood sugar level. It is questionable whether the blood sugar is really a good metabolic index of the severity of the condition. We believe that the insulin doses in our treatment schedule are adequate for the majority of cases and actually exceed the amount of insulin necessary for optimal metabolic effects.

Insulin is given intravenously for as long as the intravenous drip is administered. As soon as the patients regain consciousness, fluid can be taken orally, and insulin may be given subcutaneously.

f) Potassium Replacement
(See Therapeutic Schedule and Table 2)

Patients in diabetic coma are in a negative potassium balance which needs not always be reflected by a low potassium concentration in serum. The potassium deficit is primarily intracellular and can reach values of up to 600 meq (see Table 2). The serum potassium level in diabetic coma can be normal, low or even slightly elevated. Therefore, potassium should not be given intravenously at the beginning of therapy. As soon as the cells resume the uptake of increased amounts of glucose under the influence of insulin and store it as glycogen, they also take up potassium. The danger of hypokalemia now becomes real. As soon as the blood sugar begins to fall quickly potassium must be administered. This is usually 4 to 6 hours after beginning of treatment and can occasionally occur after as little as 2 hours. Only a small part of the cellular potassium deficit can and must be compensated for by intravenous potassium administration, since most of the patients can take potassium orally when they regain consciousness.

As a general rule, not more than 40 meq of potassium (3 g KCl) should be given in a drip in one hour.

g) Treatment with Carbohydrates

Hyperglycemic coma is by definition characterized by an excess of glucose in the blood. Insulin deficiency alone is responsible for the fact that the cells cannot use it. Therefore, administration of glucose at the beginning of the treatment of the coma is incorrect. Glucose increases hyperosmolarity, one of the main causes of the dysfunction of the brain, and makes it impossible to judge the success of insulin therapy by measuring blood glucose.

In our therapeutic schedule, the rule that the hypotonic salt solution should be replaced by a 5% glucose solution as soon as the blood sugar approaches the 250 mg% limit has proved satisfactory. Fructose therapy is still open to discussion. There is no doubt that fructose should theoretically improve the metabolic state. Liver and adipose tissue, which are really the organs responsible for ketoacidosis, can utilize fructose independently of insulin. Despite this fact, the metabolic state of the liver can not be fully normalized with fructose alone. Furthermore, fructose, sorbitol and xylitol are also rapidly converted to lactate and may lead to lactic acidosis. At this point, the liver has the tendency to convert a large part of these substances to glucose. Fructose, therefore, leads to a rise in the blood sugar, so that the effectiveness of insulin therapy can no longer be assessed by blood sugar determinations in the first hours of the treatment of the coma (NABARRO, 1955). We have recently shown that the uptake of fructose by diabetic adipose tissue is reduced roughly to the same extent as glucose uptake, so that the administration of fructose alone cannot completely normalize the metabolic disturbance of diabetic adipose tissue (FROESCH, 1965).

Nevertheless, diabetic adipose tissue has more carbohydrate at its disposal in the presence of both glucose and fructose, since both hexoses are taken up and metabolized independently of each other. Diabetic adipose tissue releases less free fatty acids when incubated with glucose and fructose together than with glucose alone. The suppressed activity of enzymes important in fat synthesis in the diabetic liver is normalized by fructose without insulin (KORNACKER, 1965).

h) Consequences of our Knowledge about the Importance of Hyperosmolarity for the Therapy of Diabetic Coma

There is no clear distinction between acidotic and hyperosmolar diabetic coma. Every patient with diabetic ketoacidosis also has a more or less pronounced hyperosmolarity. On the other hand, the pure hyperosmolar diabetic coma does not occur very frequently. The principles of treatment are the same for both forms of coma. Fluid therapy should be carried out energetically in patients in hyperosmolar coma, provided that the circulatory state of the patient permits this. We use the same hypotonic solution as for the treatment of ketoacidotic coma. It is a matter of opinion whether sodium bicarbonate should be replaced by sodium chloride.

As far as the pathophysiology of the hyperosmolar coma is concerned, the complete absence of ketosis in these patients is still unexplained. One must assume that insulin is still secreted in sufficient amounts to keep pace with the flooding of the liver with free fatty acids. Hyperglycemia must then be explained by an enormous increase of gluconeogenesis in the liver. After successful treatment of hyperosmolar coma, diet alone or combined with sulfonylureas is often adequate. This proves that the B-islet cells are still capable of secreting insulin. TEUSCHER (unpublished observation) has even succeeded in treating a patient in hyperosmolar coma with sulfonyl ureas and intravenous fluids without insulin.

However, caution must be exercised in the therapy of diabetic coma. From the electrolyte findings in Table 1, it can be deduced that the osmotic gradient between blood and cerebrospinal fluid can be temporarily reversed during the treatment with insulin when the blood sugar falls rapidly. This would cause a flow of water from the blood back into the CSF, the interstitial fluid of the brain and from there into the brain cells. Cerebral edema or swelling could arise from too vigorous treatment of the dehydrated patient. This assumption is only correct if we can apply our findings in the CSF to the interstitial fluid of the brain, a question which is not yet resolved. The formation of CSF is a very slow process compared to the formation of the interstitial fluid of the brain. It is, therefore, not astonishing that the sugar in the CSF lags considerably behind the blood sugar during the development of hyperglycemia as well as after the return to the normal state during treatment. The rapid turnover of the brain fluid also makes it unlikely that a negative osmotic difference can develop, as in the CSF. Nevertheless, this phenomenon should be further observed, since we still have no pathogenetic explanation for the fatal outcome of many cases of diabetic coma who recover first and then fall into a deep irreversible coma suggestive of cerebral edema.

7. Pathogenesis and Etiology of Diabetes Mellitus

E. R. FROESCH

a) The Inheritance of Diabetes Mellitus

Diabetes mellitus is the most common inherited metabolic disease, but its mode of inheritance is not yet known. This may be due to the fact that glucosuria is only a symptom of a generalized disease, and that other than hereditary factors determine the time at which glucose intolerance develops.

The chances are greater than 70% that the identical twin sibling of a diabetic counterpart will develop diabetes. Nearly 50% of all children of diabetic parents under the age of 40 will develop diabetes sooner or later. However, other cases of diabetics are found only in about 30% of the families of diabetes.

VALLENCE-OWEN (1965) believes that diabetes mellitus is transmitted by an autosomal dominant gene. This opinion is based on his findings that an insulin antagonist (synalbumin antagonist) could be demonstrated in the serum of all diabetics and one of their parents. These findings are fascinating because, for the first time, it appeared as if the trait of diabetes mellitus could be successfully traced from birth on. If this finding can be confirmed, the mode of inheritance of diabetic mellitus will soon be clarified. However, geneticists doubt this theory. The gene frequency in the western population is estimated to be about 22%. 5% are predisposed to diabetes, and 2–3% develop manifest diabetes. It was previously assumed that an autosomal recessive gene with incomplete or variable penetrance could best explain the mode of transmission of diabetes mellitus (STEINBERG, 1965). As long as the homogeneity of the diabetic syndrome has not been confirmed, this mode of inheritance remains doubtful. A simple autosomal recessive inheritance is also unlikely because the frequency of diabetes in children of 2 diabetic parents in whom diabetes became overt after 40 is very low (COOKE, 1966).

In a very thorough Swedish study, GRÖNBERG (1968) concluded that epidemiological knowledge about diabetes mellitus could be accounted for by 4 genetic hypotheses:

1. Autosomal dominant inheritance, in which the manifestation is diminished in the male, or in which cases of "nonessential" diabetes are more frequent in the female.
2. Autosomal recessive inheritance with the same restrictions as in 1.
3. Sex-linked dominant inheritance.
4. Multifactorial inheritance with the same restrictions as in 1.

These authors hold a sex-linked dominant inheritance as the most likely hypothesis.

NEEL (1962) thinks that a multifactorial inheritance of diabetes mellitus with 4 diabetogenic genes is more probable, based on the hypothesis of the "thrifty genotype" (NEEL, 1968).

According to this theory, it is to be expected that the severity of diabetes is dependent on whether a complete or an incomplete set of

diabetogenic genes is present. The comparison with spontaneous diabetes in animals also leads to the same conclusions. In the Chinese hamster, spontaneous diabetes mellitus occurs frequently and "juvenile" forms of diabetes as well as adult type diabetes are seen (GERRITSEN, 1967). Diabetes in the Chinese hamster is comparable to human diabetes mellitus in many ways. It is interesting to note that the mode of inheritance in this animal cannot be explained by a single gene, but rather by a set of 4 diabetogenic genes (BUTLER, 1967).

b) "Exhaustion" of the B-Islet Cells

Although a more or less complete failure of insulin secretion and hyalinosis of the islets of LANGERHANS are characteristic of the end stage of diabetes mellitus, the primary and inherited metabolic disturbance need not be situated there. It is conceivable that humoral factors could lead to a destruction of the islets of LANGERHANS. The possibility of a destructive autoimmune inflammatory process is currently being discussed as a possible cause. This point of view is not only fashionable but is supported by some observations mentioned below. One biological principle, which can also be applied to endocrinology in general, very strongly speaks against an "exhaustion" of the islet cells due to increased insulin requirements (FROESCH, 1965). When an organ, or an endocrine gland, is placed unter strain, it hypertrophies. In autoimmune diseases which lead to a decreased function of certain areas, other areas undergo temporary hypotrophy. Hypertrophy in HASHIMOTO'S goiter, a classic endocrinological autoimmune disease, is very typical. Hypertrophy of the thyroid gland quarantees a normal function for a long period of time, after which hypothyroidism develops. One could reason in favor of the "exhaustion theory" that the case of the islets of Langerhans is different: they lie as clusters of cells within a gland with exocrine function. In different animals with inherited diabetes mellitus, where the primary pathogenetic factor is increased insulin requirement, a hypertrophy of the islets is regularly found. Islet hypertrophy is particularly pronounced in akomys obesus (PICTET, 1967), and in different breeds of the obese-hyperglycemic mouse (New Zealand, Bar Harbour) (HELLMANN, 1965). In contrast, true hyperplasia of the islets has not been described in human diabetes mellitus, except in newborns of diabetic mothers. There is no proof that insulin requirement is increased in prediabetic subjects. Exhaustion of the islet cells must, therefore, be refuted as an important pathogenic mechanism because at no stage of the disease islet hyperplasia is found. The exertion of the islet cell apparatus by increased food intake and other exogenous factors can, of course, play a role in the development of islet cell insufficiency. These cells are unable to react in the normal manner with hyperplasia. A primary disorder of the B-cells of the islets is likely to be at least one of the genetic disturbances of diabetes mellitus.

c) Insulin in the Blood of Diabetic Subjects

WRENSHALL and BEST (1956) extracted little or no insulin at all from the pancreas of juvenile diabetics who had died of various causes. They also found a decrease in the insulin content in the pancreas of adult-type diabetics which paralleled the duration of hyperglycemia and glucosuria. Levels of immunoreactive insulin in the serum of juvenile diabetics are reduced and there is no increase in response to glucose (SAMOLS, 1965). In adult type diabetics the fasting values of the serum insulin may still be normal, but the rise after glucose administration is absent or reduced and delayed (SELTZER, 1967). Homozygous twins of diabetic subjects and children of two diabetic parents of the juvenile type, i.e. prediabetics by definition, already show a diminished pancreatic response to glucose before glucose intolerance becomes manifest (TAYLOR, 1967).

Overweight patients with latent diabetes often show a very special insulin response: the rise of immunoreactive plasma insulin after glucose administration is delayed, but may occasionally be even greater than normal after 2–3 hours when the blood sugar has risen to very high levels. This late insulin response can lead to hypoglycemia 3–4 hours after glucose intake (KARAM, 1963; YALOW and BERSON, 1960; SELTZER, 1967). These findings have been misinterpreted and have given rise to the erroneous expression of "hyperinsulinism in diabetes mellitus", causing some researchers to look even harder for insulin-antagonists in pre- and latent diabetes.

In our opinion such a delayed, and later on excessive, reaction of the islet cells can be explained by a relative and not yet complete decompensation of the genetically inferior islet-cell apparatus. When insulin is not released at the right time, hyperglycemia develops rapidly, stimulating the islet cells to secrete more insulin. In the diabetic, the insulin reserves are diminished and are replenished only slowly. The fact that the delayed excessive reaction may sometimes lead to hypoglycemia is consistent with this view, since extra insulin is needed to restore glucose homeostasis.

According to ANTONIADES (1965) insulin is present in the blood in a "free" and a "bound" form. The liver would convert the inactive "bound" insulin into the "free" active form during hyperglycemia. According to the research of this investigator this process would be disturbed in the diabetic. There is no evidence whatever that bound insulin contains insulin. A relationship between the partially purified nonsuppressible insulin-like activity and so-called bound insulin is more likely.

It should be stressed that the research on immunoreactive insulin in the serum has revealed many new facts about the secretory capacity of normal and diabetic islet cells. On the other hand, all hypotheses of the islet cell disturbance in diabetes based on the measurement of ILA (biological insulin-like activity) have proven erroneous.

d) Insulin Antagonists and Inhibitors

α) Endocrine Factors

Since HOUSSAY's animal experiments (1932, 1936) we know that pituitary and adrenal hormones counteract insulin. Growth hormone, glucocorticoids, catecholamines and glucagon all elevate the blood sugar. Cortisol and glucagon stimulate gluconeogenesis, whereas catecholamines and also glucagon increase glycogenolysis. Apart from this, adrenaline inhibits insulin secretion by the B-cells (PORTE, 1966) and glucose phosphorylation in the muscle cells (GROEN, 1958; MIALHE, 1965). The action of growth hormone is complex (see p. 85ff.). The hyperglycemic action of growth hormone is partly due to a stimulation of lipolysis and a consecutive increase of the plasma free fatty acids which compete with glucose for oxidation by muscle.

Diabetes can be evoked in animals by all these hormones, providing that the islet-cell apparatus has been damaged previously by partial surgical removel or chemical damage. In the pathogenesis of human diabetes mellitus there is no indication of a causal role of any insulin antagonistic hormone. The incidence of overt diabetes mellitus in acromegaly, pheochromocytoma and in Cushing syndrome is about 20–30% which corresponds to the estimated frequency of diabetic genes in the population. The endocrine antagonists are no longer believed to play a role as causal factors in the pathogenesis of essential diabetes mellitus.

β) Other Humoral Factors

VALLENCE-OWEN has been investigating the significance of the synalbumin antagonist for the past 15 years. This substance is thought to occur in increased concentrations in the serum of diabetics (1965). This antagonist inhibits the action of insulin *in vitro* on the rat diaphragm, but not on rat adipose tissue. Since the level of the antagonist is found to be elevated already in the potential diabetic, obesity before the onset of diabetes and the "exhaustion" of the islet cell apparatus occuring later could be explained by this antagonist and transitory hyperinsulinism. VALLENCE-OWEN believes that the antagonist is the B-chain of insulin bound to albumin. Conclusive evidence for this has never been presented, although there are no obvious technical difficulties in the way of testing the hypothesis.

In has been claimed that an insulin antagonist arises in the serum during diabetic ketosis. This insulin antagonist inhibits the action of insulin on the rat diaphragm, but not on rat adipose tissue (FIELD, 1956). This substance has not yet been characterized.

e) Tissue Resistance to Insulin in Decompensated Diabetes and in Obesity

Whereas the importance of the insulin antagonists in the pathogenesis of diabetes mellitus seems to be decreasing, tissue resistance to insulin has been demonstrated after prolonged insulin deficiency. Thus, the liver loses its ability to act as a regulator of glycemia soon after the development of insulin deficiency. Since we do not know how insulin acts on the liver, the failure to respond to insulin after insulin deficiency of short duration is also unexplained. If a diabetic patient's response to insulin is weak or delayed, this signifies that the liver continues to release increased amounts of glucose, or that the peripheral tissues do not take up sufficient amounts of glucose. The glucose turnover is elevated in steroid diabetes: peripheral tissues assimilate more glucose than the tissues of a healthy person, but the liver produces relatively more glucose, so that hyperglycemia can result despite an increase peripheral glucose uptake.

Muscle and adipose tissue lose their ability to take up glucose and to respond to insulin only after a long period of insulin deficiency. This has been proved by conclusive animal experiments and also by findings in man. The arterio-venous glucose difference is greatly reduced in the decompensated diabetes and cannot be acutely normalized by insulin (BUTTERFIELD, 1965; ZIERLER, 1964). When insulin is administered for a prolonged period of time, the diabetic patient's tissues are normalized and behave like those of normal subjects. We can conclude

from this that tissue resistance to insulin in the diabetic is a result and not the cause of the insulin deficiency.

The metabolism of the obese subject with normal glucose tolerance has been examined by measurements of arterio-venous catheterization. It has been shown that the muscle of the obese takes up less glucose than that of the non-obese and that its response to insulin is less pronounced (RABINOWITZ, 1962). The obese is, therefore, in a certain way resistant to insulin. The insulin values in the blood of the obese are usually higher in the fasting state and after glucose loading than in the normal, healthy person (KARAM, 1963). Both metabolic defects – reduced insulin sensitivity and increased insulin values – return towards normal as soon as the obese subject reaches his ideal weight by a reducing diet and increased physical activity. These facts cannot be explained for the time being.

f) Diabetes Mellitus as an Autoimmune Disease

Autoimmune mechanisms have been considered as pathogenic factors in several endocrine disorders, such as primary myxedema, Graves' disease and idiopathic, non-tuberculous adrenocortical insufficiency (see p. 313). Since diabetes mellitus coincides frequently with the latter condition and occasionally also with primary myxedema, it seems reasonable to look for an autoimmune process in diabetes mellitus also.

About 30% of newborns of diabetic mothers show eosinophilic infiltration of the islet tissue. Many juvenile diabetics show lymphocytic infiltration of the islet tissue within the first weeks of the onset of diabetes mellitus (VON MEYENBURG, 1940; LE COMPTE, 1958). Analogous to Hashimoto's thyroiditis, these infiltrations have been termed "insulitis". No humoral or cellular antibody has yet been demonstrated in the untreated diabetic, so that the most important piece of evidence of an autoimmune disease is still missing. It is interesting to note that antibodies can be produced by extracted insulin from one species when injected into the same species (RENOLD, 1966). In animals immunized in this way lymphocytic infiltrations similar to those found in juvenile diabetics are occasionally seen around the islet cells (LE COMPTE, 1966). These may occasionally lead to diabetes mellitus (GRODSKY, 1966).

g) Manifestation Factors: Nutrition, Obesity and Muscular Activity

The frequency of the genes for diabetes mellitus varies from population to population. The estimated frequency of diabetes depends heavily on environmental factors. This is particularly evident in various population groups in South Africa. Diabetes mellitus was quite uncommon among the Indian immigrants as long as they belonged to the proletariate of the home country, food being scarce and physical work hard. With the ascent into the middle class as merchants, food became richer and work in the fields was replaced by sitting behind a desk. People became obese and there was a "diabetes explosion". Today, up to 40% of the Indian population in certain regions of South Africa are diabetics (CAMPBELL, 1963). Similar reports have been made about Jews returning to Israel from Yemen (COHEN, 1963), and about certain North American Indian tribes (WEST, 1965).

In Europe, the decreased frequency of diabetes mellitus in wartime and the rising incidence during peace and prosperous periods are familiar facts.

The sand rat (Psamomys obesus) can be compared to the human. These rats develop diabetes when they are removed from their natural environment in the sand desert, which offers a sparse diet and free movement, into cages with restricted movement and free access to laboratory food (HACKEL, 1967).

NEEL (1962) has formulated the interesting concept of the "thrifty genotype" of the diabetic. Neel assumes that the chances of survival for the potential diabetic were favorable when periods of abundance alternated with periods of starvation, and that the diabetogenic gene has thus persisted into modern times. The 20th century of the western civilisation with its technology, increasing automatization and general abundance changed this totally. Today, the diabetic gene is still inherited because of successful treatment of diabetes.

It is futile to speculate on whether sugar or fat is the noxious agent needed for diabetes mellitus to become overt. It is rather a matter of simple overnourishment, since fat is not eaten without sugar, and conversely, sucrose not without fat. In any case, the increased use of sucrose alone cannot be held responsible for obesity and the increasing occurrence of diabetes mellitus. Patients with a hereditary fructose intolerance, for example, can become grossly overweight without any difficulty, although they do not eat mono- or disaccharides and eat nothing but starch, fat and protein (FROESCH, 1966). The remission of diabetes mellitus by restriction of calories and increased muscular activity is one of the dramatic and pleasing results that a physician can experience. They prove that excessive demands on a primarily inferior islet cell apparatus greatly accelerate the onset of the metabolic disturbance.

8. The Pathogenesis of Diabetic Angiopathy and Neuropathy

a) Diabetic Macroangiopathy

Atheromatous involvement of the large arteries (coronaries, arteries of the extremities etc.) is more frequent in diabetics than in normal subjects. Histology shows unspecific lesions, which are also found in non-diabetics. However, these lesions tend to appear earlier in young patients with diabetes mellitus. It is not yet certain whether these lesions are the sequelae of hyperglycemia. Myocardial infarction occurs frequently in patients whose diabetes was not manifest previous to the infarction. It is, of course, possible that blood sugar regulation is impaired (CLAWSON, 1949) many years before overt diabetes is diagnosed. The prognosis of myocardial infarction is worse in diabetics than in metabolically healthy subjects (SIEVERS, 1961; PARTAMIAN, 1965). We know no more about atheromatosis in diabetes mellitus than about the pathogenesis of atheromatosis in general. During hyperglycemia the artery produces sorbitol and fructose from glucose by the polyol pathway. The cells take up water and their O_2-consumption decreases. One of the noxious effects of hyperglycemia may be by way of intracellular sorbitol accumulation (MORRISON, 1972).

b) Diabetic Microangiopathy

In 1936, KIMMELSTIEL and WILSON described lesions of the glomerula specific to diabetes mellitus. They described hyaline globules in the glomerula and called it nodular intercapillary glomerulosclerosis. Later, the diffuse thickening of the basal membrane was described as a much more common sign of diabetes mellitus than the nodular form of the diabetic glomerulosclerosis. The basal membrane is focally or diffusely thickened to about ten times its normal thickness. The thickening of the basal membrane probably explains the reduced glomerular filtration. The footlike protrusions of the epithelial cells are altered in diabetes in a similar manner as in the nephrotic syndrome of different etiologies. Some authors hold that the diabetic lesion begins with a thickening of the peripheral basal membrane of the glomerula (GOETZ, 1960; SABOUR, 1962; BLOODWORTH, 1963; AZÉRAD, 1964; MACDONALD, 1964). Others localize the primary lesion in the mesangial region (MÉRIEL, 1962; FIASCHI, 1963; CAMERINI-DAVALOS, 1964).

The diabetic retinopathy also begins with a thickening of the basal membrane. Whereas the presence of a thickening of the basal membrane of the glomerula in newly discovered young diabetics has not been proven (ØSTERBY-HANSEN, 1965), SIPERSTEIN (1968) believes that such a change can always be demonstrated in the capillaries of the muscle. SÄVE-SÖDERBERGH'S findings do not support SIPPERSTEIN'S view (1966). On the contrary, these data show a good correlation between the duration of the diabetes and the thickening of the basal membrane.

The controversial question as to whether the thickening of the basal membrane and diabetic microangiopathy are due to hyperglycemia and its sequelae, or to another, independent, coexistent disease, cannot yet be answered with certainty. Even the staunch supporters of the hypothesis that another independent phenomen is involved acknowledge the fact that severe microangiopathy is seldom present before overt hyperglycemia has developed. Similar glomerular lesions to those found in the human have also been described in experimental diabetes in animals after a sufficiently long observation period (ØRSKOV, 1965).

In diabetes of long duration, microangiopathy is found in most organs, including the pancreas itself, the gastrointestinal tract, muscle, and the brain (FUNK, 1966; ANGERVELL, 1966; RESKE-NIELSEN, 1966; ZACKS, 1962).

The pathophysiological nature of the chemical changes in the thickened basal membrane is still unexplained (MUIR, 1964). According to WINEGRAD (1966), more glucose than normal could be metabolized by the glucuronic acid pathway. The basal membrane of the glomerula contains glucose and galactose bound to collagen and in the form of sialiofructohexosamine glycane (DISCHE, 1965). It is possible that the synthesis of these carbohydrate-containing components of the basal membrane is dependent on the activity of the glucuronic acid pathway, which is increased in diabetes.

c) Present Knowledge

The pathophysiology of the acute metabolic disturbance in diabetes, the diabetic coma, is more or less well understood. The intermediary metabolism and its regulation and disturbances are relatively well known since they can be investigated in the experimental animal. Relative or absolute insulin deficiency leads to hyperglycemia and glucosuria, because cell membranes are impermeable to glucose in the absence of insulin, so that glucose uptake ceases. The insulin action on hepatic glucose production is also lost: in spite of hyperglycemia, the liver continues to release glucose into the blood, thereby increasing hyperglycemia. Adipose tissue liberates free fatty acids into the blood from

triglyceride stores. Triglyceride lipase is no longer inhibited by insulin and the free fatty acids cannot be reesterified. Free fatty acids are oxidized to keto acids in the liver, which flood the organism and eventually lead to ketoacidosis. Insulin and the intravenous administration of hypotonic solutions can correct all acute diabetic disturbances. The pathogenesis of insulin deficiency and of the diabetic complications are much more difficult to investigate and are still not clear. Possibilities and hypotheses have been discussed. Definite conclusions have not yet been reached. Diabetes mellitus is inherited, but the exact mode of inheritance is still not known. How and where the diabetogenic gene(s) act, is still a mystery. A disturbance in the biosynthesis of insulin or its secretion must be present. This results ultimately in an inability of the islet cells to secrete sufficient amounts of insulin.

D. Morphology of Diabetes Mellitus

H. STEINER

1. General

The morphology of diabetes embraces three main forms which have some common traits and some particularities 1. labile juvenile diabetes ("lean diabetes"), 2. stable, mild diabetes with obesity, and 3. pancreatic diabetes.

Although large series allow determination of typical morphological patterns quantitatively and qualitatively, in an individual case it is often not possible to make a definite diagnosis on the basis of morphological criteria, since most of the findings can also occur, although much less frequently, in non-diabetics or prediabetics. Conversely, normal morphology may exceptionally be present in the diabetic (e.g. normal B-cell mass in individual cases of stable mild diabetes).

2. The Pancreas and the Islets of Langerhans

a) The Exocrine Pancreas

The pancreas, as a whole, is often considerably enlarged in adult obese-type diabetes, but as a rule, the increase in volume is due to deposition of fatty tissue parallelling the generalized obesity. There may, however, be a net reduction of the parenchymatous mass in such cases. Generally, pancreatic weight does not deviate from normal in cases of acute juvenile diabetes, whereas the weight of the pancreas is often greatly reduced during the chronic course of the disease (GEPTS, 1965), although increased weight is sometimes observed even in these cases.

The total islet volume also shows the same wide variation among individual cases as the proportion of islet volume to the total pancreas does (Fig. 9). These volumes are difficult to estimate, and careful planning of the measuring technique is required to exclude erroneous interpretation and false results. On average, the islet-cell tissue of the diabetic is reduced by over 50% in comparison to the volume in the healthy subject of the same age. However, the proportions may be within absolutely normal limits. This loss of islet parenchyma is definitely less pronounced in diabetes of the obese type than in juvenile "lean" diabetes (Fig. 9). This mass decrease in the latter group is demonstrable shortly after the onset of the disease, even if the islets are large and very active B-cells can be detected. The reduction is much less marked, if present at all, in pancreatic diabetes, where definitely hyperplastic islets can often be seen, in contrast to essential diabetes. This also applies for pancreatic cirrhosis associated with hemochromatosis (HEDINGER, 1953). In addition to the severe fat infiltration already mentioned, which occurs mainly in diabetes of the obese type, arteriosclerosis, a focal or diffuse interacinous fibrosis and lymphocytic infiltrations of the pancreas are very common in chronic diabetes. Foci of acute pancreatitis can be observed not uncommonly in acute cases (LAZARUS and VOLK, 1962).

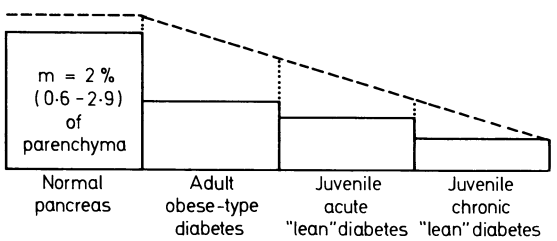


Fig. 9. Percentage proportion of islet parenchymal mass to total pancreatic parenchyma in different forms of diabetes and in healthy subjects. Schematic representation. The dashed slanted line indicates the upper range limit (left border lining of the squares)

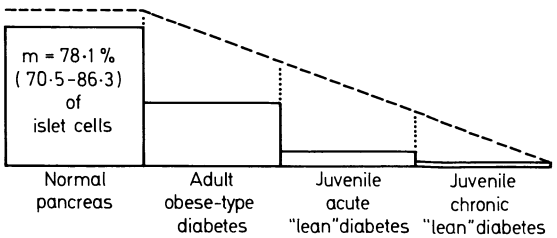


Fig. 10. Proportion of B-cells to the total mass of islet cells. Schematic representation. Strokes indicate the upper range limit (left border lining of the squares)

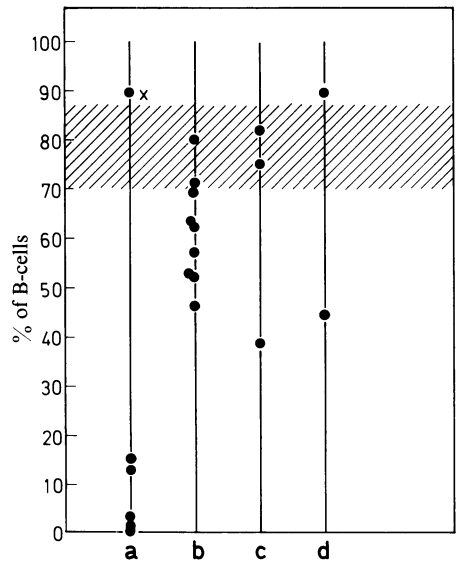


Fig. 11. Proportion of B-cells in the islets of Langerhans in 20 diabetics (staining after GOMORI-RUNGE). The hatched area indicates the range of healthy nondiabetics. (According to VON WATTENWYL, 1964)
a Labile juvenile diabetes; *b* stable adult obese-type diabetes; *c* peracute juvenile diabetes; *d* insulin-resistent diabetes; *x* 45-year-old uremic male with clinical juvenile diabetes of 16 years' duration: To be classified morphologically into the group of adult obese diabetes (insulin determinations not performed)

b) The Islet System

Deviation of the ratio of A-cells to B-cells in favor of the A cells is a fairly constant *quantitative change* (Figs. 10 and 11). This is due to a reduction of B-cells and not to an absolute increase of A-cells. This *relative* A-cell increase is due to the glucagon-producing A_2-cells in such cases. The decrease of B-cells is again very marked in chronic juvenile diabetes. In the acute form of the disease, the B-cells are usually reduced in number, but at this stage they still show signs of high secretory activity. The B-cells are often reduced by 40 to 50% of normal in adult obese-type diabetes, but individual variation is very wide (Figs. 10 and 11). Sometimes, small (newly formed) islets consisting almost entirely of strongly granulated B-cells, are seen in these patients (Fig. 12). In contrast to these findings, quite definite increases in the islet and B-cell masses have been observed in *animal experiments on the healthy animal* receiving continuous glucose infusions (KINASH and HAIST, 1954). Massive islet-cell proliferations can also be observed in several types of animals after immunization with heterologous insulin. The following findings are present in *newborn infants of diabetic women:* true hypertrophy of the islet

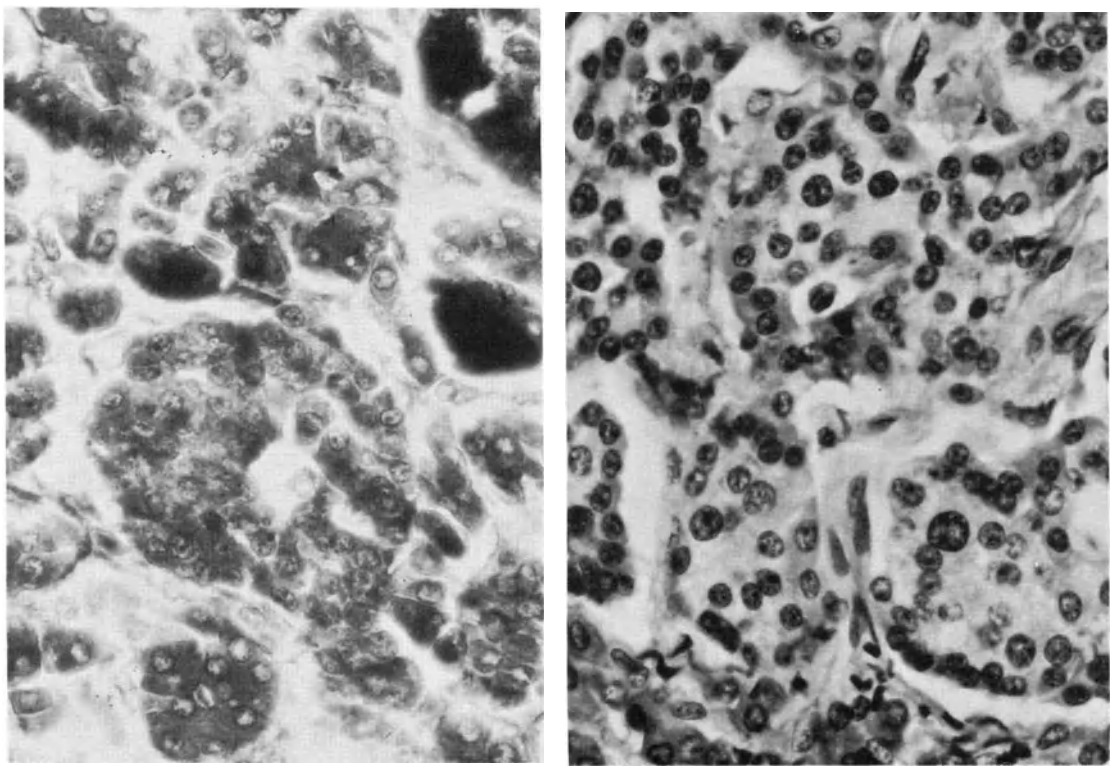


Fig. 12. Small islets of Langerhans with richly granulated B-cells (new formation) and large islets with strongly degranulated B-cells and nuclear hyperplasia in a 60-year-old female diabetic with stable, mild diabetes. Stain: Gomori-Runge; Scale 470: 1

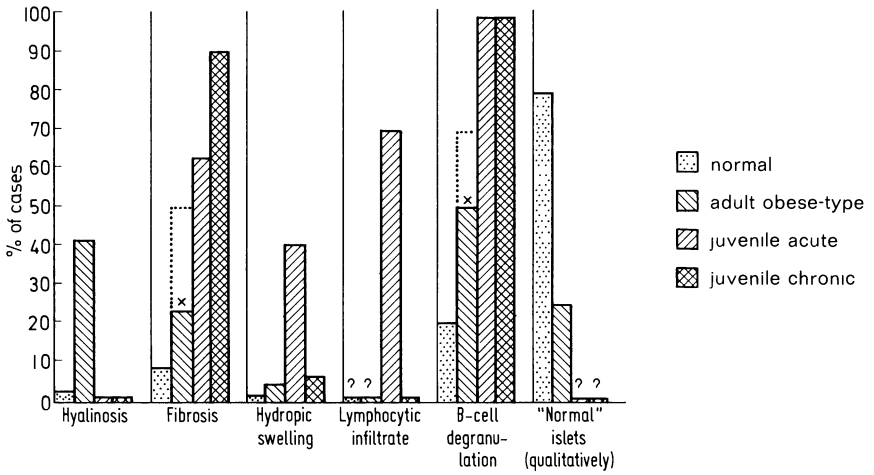


Fig. 13. Average frequency of typical histological changes in the islets in diabetics and healthy subjects. Schematic representation taking different sources into consideration. (Particularly WARREN and LE COMPTE, 1952; SEIFERT, 1958; LAZARUS and VOLK, 1962; GEPTS, 1965)

apparatus with polynesia (many islets), maccronesia (large islets) and a strong predominance of B-cells, so that the weight of the islet apparatus can be as much as 10% of that of the total pancreas. These changes have also been observed in children of women with latent diabetes. Pharmacologically-induced hyperglycemia (e.g. with corticosteroids) in animals causes a significant increase in the B-cell mass and islet volume (the latter increasing up to 4 times more than in controls; HELLERSTRÖM, 1963). The figures for an adult with steroid diabetes or acromegaly correspond closely to those for subjects with diabetes of the obese type. From this it can be deduced that diabetes mellitus only develops where a genetic predisposition does exist.

The islet tissue also undergoes numerous, but mainly nonspecific *qualitative changes*, which can differ with the various diabetic groups (Fig. 13). The majority are probably due to secondary factors (? vascular). *Lymphocytic infiltrations* of the islets of Langerhans (von Meyenburg's insulitis) (Fig. 14) is a very typical change in acute juvenile diabetes. It has a striking similarity to inflammatory features in an autoimmune disease (see also p. 762).

The B-cells are very active in the first phase of juvenile diabetes. The cells are mainly totally degranulated and reflect a high degree of synthetic activity which can be detected by the accumulation of Weichselbaum granules (structures in cytoplasm of very active B-cells developed through aggregation of ribonuclein, stainable with toluidine blue, and eliminable with ribonuclease), and enlargement of the nuclei.

Hydropic changes not uncommonly occur in the cytoplasm of B-cells in acute juvenile diabetes, where vacuolizations and glycogen infiltrations arise (GEPTS, 1965). These changes are very rare in chronic forms of diabetes. The few B-cells present in juvenile diabetes lasting for many years are atrophic, and B-cell granules are hardly demonstrable. Islet hyalinosis

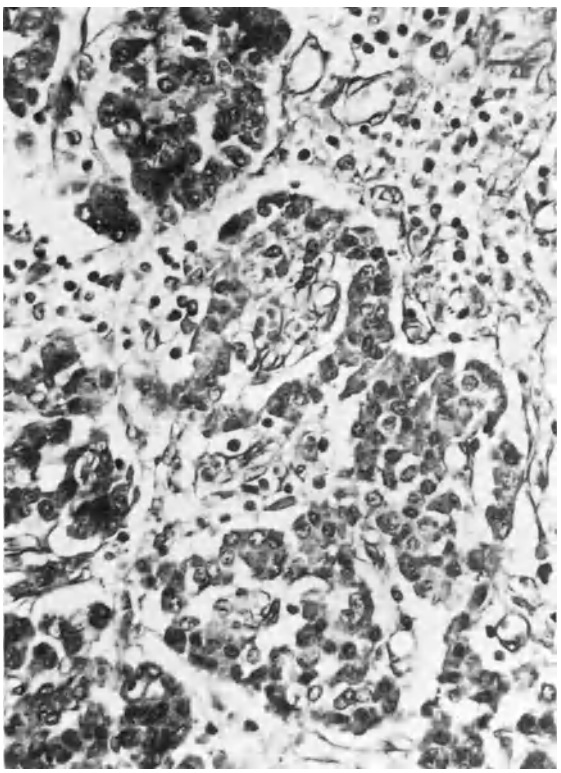


Fig. 14. Lymphocytic insulinitis and periinsulitis in a 9-month-old boy with peracute diabetes lasting 4 days. Insulin treatment started few hours before death. Stain: Gomori-Runge; Scale 350: 1

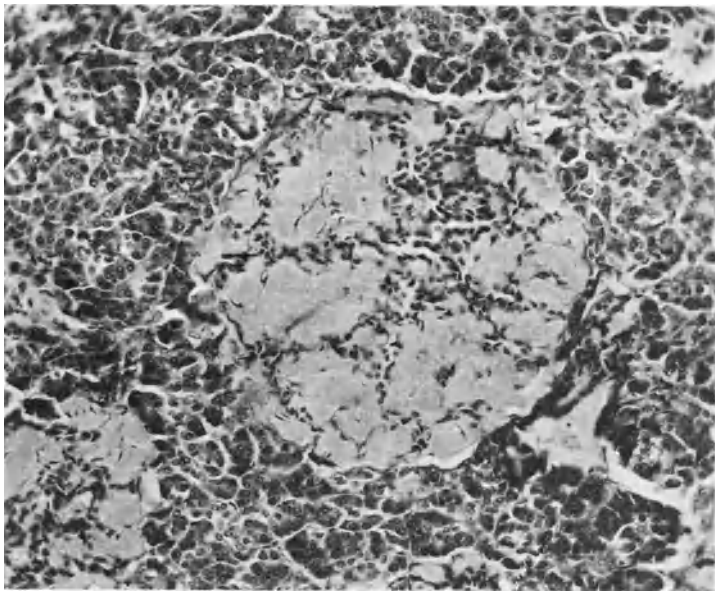


Fig. 15. Hyalinosis of the islets of Langerhans. Stain: hematoxyline-eosin; Scale 120: 1

is only rarely found in chronic lean diabetes, but is frequent in the obese type (Fig. 15). This change increases in frequency in the latter group with increasing age and is not uncommon in non-diabetics, whereas it can seldom be detected in the former group even after diabetes of 30 years' duration. The severity is by no means parallel to the degree of severity of the diabetes. On the other hand, *fibrosis of the islets* is a common finding in both groups. Investigations in the past few years have shown that this amorphous hyaline infiltration is due to fine fibrils of amyloid deposited between the basal membranes (LACY, 1964). Such amyloid deposits are, as a matter of interest, frequently encountered within B-cell insulomas (PORTA, 1962). The B-cells of obese diabetics are usually well granulated. The insulin content of the pancreas of these patients is relatively large, but the threshold for the release of insulin is higher.

To summarize: the manifold changes of pancreatic morphology are a reflection of an extensive and progessive hypofunction of the islet apparatus after initial high activity in juvenile diabetes. In the obese-type diabetes, however, there is only a moderate quantitative and qualitative insufficiency of the B-cells which, as in pancreatic diabetes, is compensated in part by increased activity of the cells present. The content of granules is high in this stable form of obese-type diabetes, but the threshold for insulin secretion is greatly raised. Thus, in addition to influences leading to reduction of B-cells, there are other noxious agents which impair the action of insulin (see p. 760). For these reasons, diabetics often require a larger amount of insulin than patients who have undergone pancreatectomy (see pp. 749 and 808).

3. Primarily Vascular Concomitant and Subsequent Symptoms (So-Called Late Syndrome)

a) *Arteriosclerosis of the great vessels* is more severe in diabetics and arises earlier than in nondiabetics. The lesions, however, are seldom specific. Thus for example a picture familiar to a clinician – Mönckeberg's media sclerosis – may also be encountered in the elderly nondiabetic. As a rule there is endothelial proliferative thickening through colloid, hyaline substances, deposition of lipids and splintering of the tunica elastica. These morphological changes can hardly be differentiated from those in the nondiabetic.

b) *Arteriolosclerosis* is also a nonspecific change which occurs more frequently in diabetics than in nondiabetics. It is often associated with hypertension.

c) *Diabetic microangiopathy* is a very characteristic change which must be held directly or indirectly responsible for many of the so-called late complications. It is a proliferative endarteritis with deposits positive to PAS (period acid, Schiff) and negative to colloidal iron. This contrasts to the reactions in common arteriosclerosis. The elastic internal membrane is usually intact in the arterioles. Not uncommonly there is an increased number of perivascular mast cells. The initial lesion is a pronounced thickening of the basement membrane in particular. This change appears as PAS-positive

homogeneous lesions (FUNK, 1965) in the capillaries and pre-capillaries. Antiimmunoglobulins can be fixed immuno-histochemically in these lesions (LARSSON, 1967). According to certain authors, this homogeneous thickening of the basement membrane can be found in the muscle capillaries in up to 50% of healthy subjects born of two diabetic parents (SIPERSTEIN, 1967). Others did not observe this finding. POMETTA, for example, was unable to reproduce several expected results.

Microangiopathy is dispersed in many organs. The lesions seem to lead to the manifest disease particularly when tissue function is lost in the end organs due to the reduction of blood supply. These organs are often fibrotic. Permeability of the vessels is increased due to impaired metabolism and thickening of the membrane. This in turn leads to further characteristic changes (see below).

Renal changes are of considerable practical importance:

d) *Diabetic nephropathy.* In addition to the changes in the small vessels, *glomerular sclerosis* is of particular importance (Fig. 16) in the kidney of diabetics. This nephropathy may take the form of nodular thickening in the intercapillary spaces, or of a diffuse PAS-positive thickening of the basement membrane, or of a combination of both. These ultrastructural changes can be detected quite early. Changes in the intercapillary spaces seem to precede the thickening of the basement membrane as a rule (KIMMELSTIEL, OSAWA, and BERES, 1966), and development in juvenile diabetes seems to be dependent on the duration of the disease. More recent investigations also indicate that the development of glomerular sclerosis is connected with the arteriolosclerosis of the kidneys (FISHER, 1967). Glomerular sclerosis in the nodular and diffuse forms is a very common complication of juvenile diabetes of long duration, and is the main cause of death in this group. In fact, nodular glomerular sclerosis has only very rarely been observed in nondiabetics, and may be considered as a specific diabetic complication. The importance of other forms of nephropathies is considerably less compared with that of glomerular sclerosis. The *Armanni-Ebstein* nephropathy (RITCHIE and WAUGH, 1957) con-

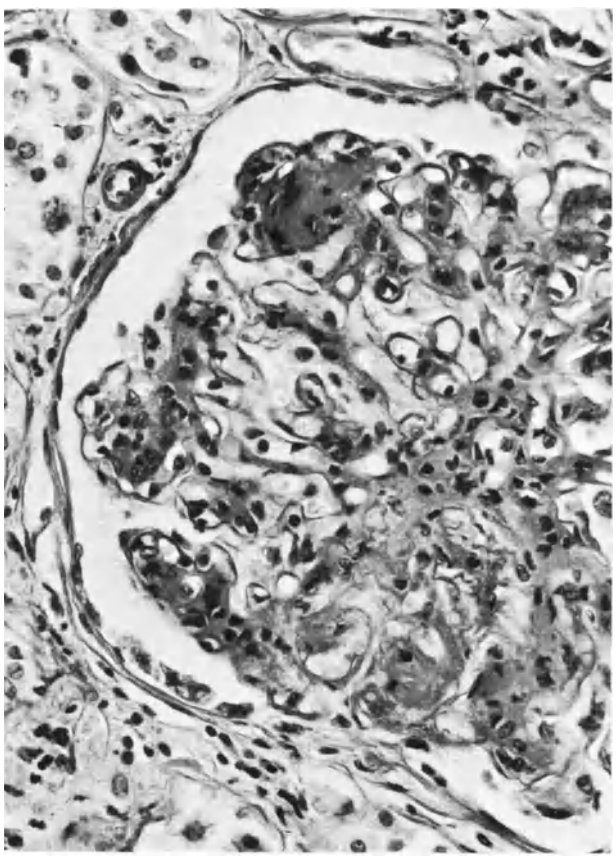


Fig. 16. Kimmelstiel-Wilson glomerular sclerosis with severe hilifugal, PAS-positive thickening of the glomerular loops. Stain: PAS; Scale 450: 1

sists of an extensive vacuolar deposition of glycogen in the cytoplasm of the contorted proximal part of the renal tubule. This change is observed particularly often in cases with severe changes in sugar metabolism leading to diabetic coma. It can also be produced experimentally in animals through severe hyperglycemia.

Acute papillary necrosis is a grave complication of pyelonephritis often found in diabetics (see p. 797). It is an ischemic necrosis of the renal papilla. It often leads rapidly to anuria. The lesion is not specific to diabetes.

diverticula of the capillary walls of the venous limb which are often thickened by hyaline masses containing lipids (Fig. 17). See p. 792 for the influence of hypophysectomy on diabetic retinopathy.

Refer to appropriate textbooks for treatment of other morphological changes in the eyes (in the iris, conjunctiva and lens) in diabetes mellitus.

f) The initial processes in *diabetic neuropathy* are partly attributed also to metabolic disorders due to changes in vascular permeability. The microangiopathy in small perineural vessels can

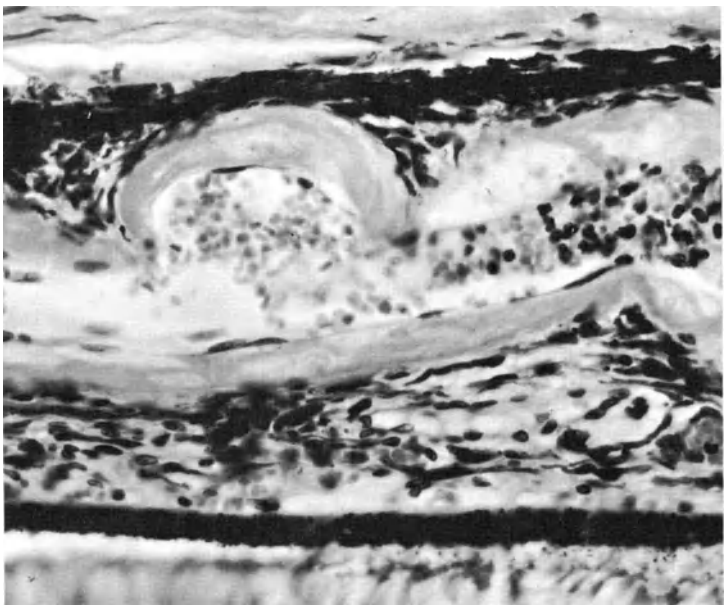


Fig. 17. Severe arteriosclerosis with aneurysmal protrusion of a vessel in the membrane choriocapillaris of the left eye. Stain: Van Gieson; Scale 250: 1. Same case as in Fig. 16

e) *Diabetic retinopathy*. This complication can be considered as a result of angiopathy. It is now the most common cause of blindness in the Western hemisphere. The early change is thickening of the basement membrane. Morphological findings include variations in the caliber of the vessels, microaneurysms, hard and soft exudates, hemorrhagia and *proliferative retinitis*. The last change can be described morphologically as capillary proliferations in hemorrhagic foci and ischemic areas. The soft exudates (cottonwool foci) are due to changes probably developed primarily because of metabolic disturbances which can partly be accounted for by the hypoxia secondary to capillary changes. The hard exudates are localized in the outer reticular retinal layer and represent serum exudated from the vessels (DIEZEL and WILLERT, 1963). These hard exudates can be absorbed under certain conditions. The microaneurysms are first be considered to play a significant role in the advanced form due to the poor vascularization of the tissue. Thickening of the basement membrane is the first detectable morphological sign of the disease. In addition to the classic form of *symmetric neuropathy*, the *ischemic mononeuropathy* is characterized by multifocal small infarctions of the nerves (circumscribed ischemic necroses). The vascular disorder in this disease is probably localized outside the nerve in small arterioles. Diabetic neuropathy also involves nerves of the autonomic system.

g) The microangiopathy of the heart in particular (myocardial infarction), the extremities (diabetic gangrene) and skin (diabetic dermatopathy, necrobiosis lipoidica) are of clinical importance. The morphology of the changes in the small vessels cannot be differentiated from that of the changes in other organs.

With the exception of Kimmelstiel-Wilson glomerular sclerosis, all these changes have in common that they also occur, though much less frequently, in nondiabetics. The changes are thus morphologically nonspecific. Thickening of the basal membrane is often the only finding in the initial stage. The raised permeability of the vessels resulting from this is held partly responsible for the morphological consequences. The microangiopathy and ordinary arteriosclerosis are usually localized in several organs. For example, retinopathy is almost always associated with a glomerular sclerosis.

h) *Hypophysis and diabetes mellitus.* It is of interest that microangiopathies are partly reversible by *hypophysectomy* (see Chap. V, p. 87). The thickening of the basement membrane in particular disappears. On the other hand, the arterioles remain unaffected.

For this reason, the interest in the hypophysis is evident. This gland is closely linked with sugar metabolism through the various hormone systems. A more or less significant infarction of the hypophysis is not a very uncommon finding in diabetes. The acidophils (producers and storers of growth hormone and prolactin) are greatly increased in the hypophysis particularly in juvenile diabetes with severe hypoglycemia. On the other hand, no significant deviation in the cellular picture is seen in diabetes in the elderly where the hyperglycemia is not severe (STEINER, 1965). (Juvenile diabetes: 59 ± 11.9% acidophils; adult obese-type diabetes: 43.5 ± 13.8%; normal: 34.8 ± 8.2% in the male; and 36.8 ± 6.3% in the female).

4. Other Common Morphological Changes in Diabetes Mellitus

Most changes are not specific to diabetes mellitus, as has been shown above, and can also arise in other metabolic disorders.

a) *Liver changes.* Glycogen inclusions and swelling of the nuclei of hepatic cells are often found in severe diabetes. These inclusions also develop in pancreatic diabetes produced experimentally with alloxan. Intensive investigation showed nuclear glycogen in the liver in 80% of a group of diabetics, and in 53% of the control group (SELLYEI and WALTON, 1969). Liver enlargement is not uncommon and is usually due to obesity. Enlargement associated with a large deposition of glycogen (Mauriac's syndrome) is seen in particular in the poorly controlled juvenile type. Liver enlargement and glycogen depots disappear again when the diabetes is restabilized.

b) *Lesions due to treatment.* A whole series of changes is directly or indirectly connected to treatment of the disease: complications due to development of resistance and allergy, typical appearance of lipodystrophy (disappearance of fat in the region of the injections), and local fibrosis and swelling in the regions of the injections (see p. 790).

c) *Infections.* These differ only in frequency and course in the diabetic. The morphological picture is hardly different from that of the same infection in the nondiabetic.

E. The Clinical Features of Diabetes Mellitus

G. R. CONSTAM

Diabetes mellitus is defined as a chronic metabolic disorder leading to incomplete utilization of the absorbed carbohydrates and to a rise in the blood sugar, usually associated with the excretion of pathologic amounts of glucose in the urine.

It is to be expected that better knowledge about the pathogenesis and the etiology of this disease will soon necessitate alterations in the definition, in the diagnosis and in the differential diagnosis of diabetes millitus.

1. Incidence

Diabetes mellitus occurs at any age and in all races. The diabetes *morbidity* was estimated to be 0.7% before the First World War, and 1.5% in 1950, in Europe and the U.S.A. These figures were based on the examination for urinary sugar in whole population groups. When the urine was examined for sugar 2 hours after an English breakfast, the proportion of diabetics rose to 4%, and even to 12% when extra sugar was given. In a large American city, the glucose tolerance test revealed a 15% incidence of diabetes, and in Mexico 13% of the age group over 60 years were diabetic. The uncommon occurrence of diabetes in younger age groups can be explained by the fact that the disease is very often still in the latent phase at this age.

Statistics from South Africa about Indian immigrants into Natal are not at all in keeping with the above figures. It was found that diabetes mellitus occurred 10 times more frequently in these Indians than in their original homeland, and that 40% of the population suffered from diabetes after 20 years of immigration.

All authors, including the experts of the World Health Organization, are of the opinion that the incidence of diabetes is increasing considerably. SCHÖFFLING (1965) found the same in Central Germany, OTT (1957) in

Switzerland. The Health Authorities of U.S.A. estimated the number of cases of overt diabetes to be around 2 million in 1949, and to be 3 million in 1964. They expect to have 4 million cases by 1970. It is not known to what extent the determined *increase* in *diabetes morbidity* represents a real increase and not only the result of greater life expectancy and of improved methods of investigation.

See p. 759 about etiology, inheritance and environment.

The relations between *diabetes mellitus and trauma* are of interest to both the physician and the specialist. To produce diabetes mellitus experimentally in an animal, the greater part of the pancreas must be removed, in the dog, for example, 9/10 of the pancreas. Experience gained from pancreatectomies confirms this for man. An accident involving the region of the pancreas can only cause diabetes if most of the organ is destroyed. Such a lesion must also result in insufficiency of the exocrine secretion, and such severe injuries always are fatal. Cases of diabetes caused by traumatic damage to the pancreas are therefore very unlikely.

Theoretically, sclerosis of the pancreas can occur as a result of injury or pancreatitis, but, so far, this has not been proven histologically. *Traumatic pseudocysts* of the pancreas do not cause diabetes, whereas pseudocysts after pancreatitis, calcifying pancreatitis and the late stages of mucoviscidosis (see p. 809) can.

Cerebral concussion, intracranial hemorrhage and, less often, injury of bones or soft tissues, can be followed by hyperglycemia and glycosuria. This is a temporary extra-insular *stress glycosuria* (see p. 774). Despite the enormous number of cranial injuries during the last wars, there was no increase in diabetes mellitus. Not a single case of diabetes of traumatic etiology was found by VON NOORDEN in 20000 diabetics, or by UMBER in 7000 patients or by JOSLIN in 40000 cases.

Even if trauma has been dismissed as the cause of diabetes, it can, however, act as a releasing factor, i.e. it can cause potential, chemical or latent diabetes to become clinical. In addition, reduced physical activity, overeating, infections, anxiety, worries or pain can cause a subclinical diabetes to become manifest. Probably, these cases would have developed clinical diabetes even without an accident (or releasing factors) if the subjects concerned had lived long enough.

For the same reasons, *deterioriation* of pretraumatic diabetes is also possible. On the other hand, preexisting diabetes can also influence the *results* of an accident, diabetics being predisposed to infections and slow healing processes.

Tumors as well as inflammations of the pancreas must be very extensive before they produce diabetes. In *acute pancreatitis*, the glucose tolerance is only temporarily diminished. The majority of pancreatic necroses as well as tumors of the pancreas progress without developing diabetes. *Chronic pancreatitis* with or without *calcifications* and formation of *calculi* is often considered a cause of diabetes. *Arteriosclerosis* can lead to hyaline degeneration and to fibrosis of the islets of Langerhans. The connection with diabetes is not clear, since we do not know if the latter is a result or the cause of vascular changes.

The development of hyperglycemia and glycosuria has been observed during treatment with *adrenocortical, anterior pituitary* and *thyroid preparations*, with *diuretics of the benzothiadiazine group*, with *chlorthalidone*, and also after severe extensive *burns* treated with a high caloric diet. Probably a previously potential diabetes has been activated in these cases.

2. Prevention

Prevention is hampered by our lack of knowledge about the cause of the disease and by our inability to diagnose it at an early stage. For the time being, the predisposition to diabetes mellitus cannot be detected clinically with sufficient certainty.

Usually, married couples are advised not to have children when both partners have overt diabetes. In practice, however, the majority of cases of conjugal diabetes are not detected until after they have already had children or even grandchildren.

Table 3. Classification of diabetes mellitus (see also Table 4)

	Causes
1. Essential, *familial* primary diabetes:	Unknown, perhaps inheritance and provocative factors
2. Non-essential *secondary* diabetes:	
a) Insular insufficiency diabetes:	Total or subtotal pancreatectomy, tumors, inflammations of the pancreas, mucoviscidosis, hemochromatosis.
b) Extra-insular diabetes:	Adrenaline- (stress-stimulating) diabetes, acromegaly, Cushing's syndrome, hyperthyroidism, Conn's syndrome
3. *Uncommon special forms of diabetes:*	
Lipo-atrophic diabetes (LAWRENCE)	
Myatonic diabetes (PRADER, LABHART, WILLI)	

Table 4. Stages of *essential* diabetes (World Health Organization 1965) (see also HAMURI, 1967)

1. *Potential diabetes:*
   Metabolic disturbance not detectable with the present-day laboratory methods. Diagnosis should be suspected in homologous twins if one is already diabetic, in children with both parents diabetic, children with father or mother and a sibling diabetic or cases of diabetes in the family of the non-diabetic parent (grandparent, uncle, aunt, cousin), in women delivered of a baby with a birthweight of 10 pounds or more or stillbirths with hyperplasia of the islands of Langerhans in the absence of rhesus incompatibility.
2. *Latent diabetes:*
   a) Normal oral glucose tolerance test which, however, had been abnormal under stress or when obese.
   b) Cortisone-glucose tolerance test abnormal.
3. *Asymptomatic = subclinical = chemical diabetes:*
   Oral glucose tolerance test abnormal, but no other diabetic symptoms.
4. *Clinical or overt diabetes:*
   Oral glucose tolerance test abnormal, diabetic symptoms or complications.

The diagnosis of a *pre-diabetes* can only be made in retrospect, when potential diabetes has progressed into latent, asymptomatic or clinical diabetes.

Table 5. Classification of essential diabetes (WHO)

	Age of manifestation	Characterization
a) Childhood (infantile) diabetes	0–14 years	Needs insulin early
b) Juvenile diabetes	15–24 years	Majority need insulin at an early stage
c) Adult diabetes	25–64 years	Begins less abruptly, needs for insulin vary
d) Diabetes of old age	65 years and over	Often long periods without insulin therapy

Table 6. Differential diagnosis of melliturias

A. *Normoglycemic*
   1. Renal glycosuria
      a) Primary, sometimes familial glycosuria with reduced renal threshold of varying degree
      b) Secondary renal glycosuria through damage of the proximal tubules
         α) Familial: de Toni-Fanconi-Debré syndrome
         β) Acquired
      c) Glycosuria during pregnancy
      d) Neonatal melliturias
   2. Glycurias
      a) Pentosuria (see p. 775)
      b) Fructosuria (see p. 775)
      c) Galactosuria (see p. 775)
      d) Lactosuria (see p. 775)
      e) Saccharosuria (see p. 776)
      f) Mannoheptulosuria (see p. 776)

B. *Hyperglycemic* (sometimes only of short duration)
   1. Primary essential diabetes mellitus, predisposition inherited
   2. Non-essential, secondary diabetes mellitus
      a) Pancreatogenic
         α) Acute pancreatitis
         β) Fibrosis of the pancreas
         γ) Pancreatectomy
         δ) Hemochromatosis
         ε) Hunger-diabetes (transitory)
      b) Extra-pancreatic
         α) Adrenaline ("irritant" "stress") glycosuria
         β) Acromegaly
         γ) Cushing's syndrome, glucocorticoid administration
         δ) Hyperthyroidism
   3. Oxihyperglycemic glycosuria, tachyalimentation, pseudo-diabetes
      a) Accelerated absorption
      b) Delayed or disturbed formation of glycogen

The alimentary cyclic glycosuria can be due to 1. early diabetes mellitus, 2. renal glycosuria, 3. oxihyperglycemia. The glycosuria in pregnancy can be the consequence of 1. early diabetes, 2. a fall in the renal threshold through increased glomerular filtration rate.

Prevention of diabetes tries to remove factors promoting the manifestation of the disease. Among these, avoiding overweight through increased muscular activity and corresponding adjustment of nutrition are of the greatest importance. Active foci of infection, especially of the biliary tract and of the pancreas must be eliminated, mental stress whenever possible avoided. Women with a family history of diabetes should not have more than two to three pregnancies.

Recognizing diabetes in its early stages is of great importance, since it seems possible that early onset of therapy can simplify the treatment and improve the long-term prognosis.

It is still controversial whether electronmicroscopic or histochemical vascular changes can occur prior to demonstrable metabolic disturbances and thus indicate potential diabetes. In the future, potential diabetes may become detectable by metabolic or morphological deviation which is not yet known.

3. Course of Diabetes Mellitus

The development of diabetes from the potential to the latent and finally to the clinical stage of the disease is rapid in some people and slow in others. Symptoms and clinical features may vary considerably, depending on whether the disease arose during growth or after maturity or at old age. This difference may be so great that some authors believe two different diseases are involved. This seems unlikely in view of the

fact that different forms can arise in the same family and that one form may change into the other. We, therefore, think that there is only one disease with different rates of evolution. When the disease develops rapidly, it becomes overt before or shortly after the termination of growth, leading relatively soon to failure of the islet system and to insular-insufficiency diabetes. On the other hand, when the disease develops slowly and remains for some time in the stages of latent or chemical diabetes plasma insulin may in some cases even be increased (see p. 761). In clinical diabetes *relative islet insufficiency* usually progresses so slowly into *absolute islet insufficiency* that the majority of diabetics die before reaching this stage.

The 2500-year-old classification of the "fat/sthenic" and the "thin/asthenic" (insulin-deficient) diabetes is for didactic reasons still justified today. The former type is predominantly found in the elderly, the latter usually in younger patients, in thin people mainly before their 46th year.

a) Subjective Symptoms

The *subjective symptoms* are: fatigue, polydipsia, polyuria, loss of weight, the last three being called the "cardinal symptoms of diabetes". In children weight loss despite polyphagia is striking. Before the disease becomes overt, some patients complain of weakness and tremor 4 to 5 hours after a meal. This *reactive hypoglycemia* (see p. 816) disappears after the ingestion of carbohydrates, which explains the increased longing for sweets, particularly for chocolate.

In small children, the reappearance of enuresis, in the adult a sudden development of myopia, and in the elderly dizziness are suggestive of diabetes.

The diabetes of elderly overweight persons causes little trouble for a long time and, with few exceptions, shows little tendency to ketoacidosis. On the other hand, during growth and in normal or underweight elderly persons polyphagia may rapidly change into loss of appetite, nausea, then vomiting, and later somnolence, possibly followed by coma.

b) Objective Symptoms

In the "*fat/sthenic*" *diabetes* rubeosis of the skin, especially of the face, disturbances of the coronary, peripheral and cerebral circulation, of the renal and central nervous systems are common. The retina may also be affected, and cataracts may be present. Glycosuria may be absent even in the presence of hyperglycemia which varies less quickly than in the "thin" diabetic. The tendency to ketosis and ketonuria is slight. The diabetes responds well to weight reduction and to sulfonylurea derivatives, at least at the onset of the disease, which is only slightly response to insulin. This form of diabetes is benign only in so far as its tendency to acidosis is concerned, and malignant on account of its susceptibility to vascular complications. The main goal of its therapy is to prevent, reduce or treat these vascular disturbances.

There are exceptions to this rule, and the "fat" diabetes can change its character within a few days, usually under the influence of some stress, and even develop ketoacidosis.

The "thin/asthenic" diabetic shows severe glycosuria at an early stage, sometimes even with moderate hyperglycemia. The blood sugar fluctuates, there is a strong tendency to ketonemia and ketonuria. It is sensitive to insulin, whereas the sulfonylurea derivatives are only temporarily effective in the very early stages. Here, the first purpose of treatment is to prevent the occurrence of ketoacidosis, the second the prevention of the long-term diabetes syndrome.

4. Diagnosis and Differential Diagnosis

For the time being, our diagnosis is still based on the demonstration of a *delayed or reduced ability to assimilate carbohydrates* in the presence of normal gastrointestinal function. This impaired carbohydrate assimilation is perhaps not the cause, but already a consequence of diabetes. Measuring carbohydrate assimilation by glucose tolerance tests does not furnish a sharp dividing line between diabetes and non-diabetic conditions. Thus, in some cases diagnosis has to be deferred and may be confirmed only after long observation and repeated investigations. In the early phases, the metabolic disorder appears irregularly, hyperglycemia with or without glycosuria may disappear spontaneously and reappear only after months or many years.

If the evidence is sufficient, we are able to diagnose diabetes, but in the absence of signs and symptoms, we can never exclude definitely the presence of potential diabetes.

The diagnosis of diabetes mellitus is based on the *family and personal histories, a thorough general examination and the determination of glucose in urine and blood.* Diabetes in a near relative strengthens the suspicion of diabetes and indicates diabetes mellitus even if a slight abnormality of the carbohydrate assimilation has been found which by itself would not be sufficient for diagnosis.

In the *typical personal history*, there are or were cardinal symptoms present at least in severe cases. The *clinical examination* of the diabetic patient has to exclude other causes for the symptoms, and to detect any late symptoms or complications.

Routine examination for glycosuria most often leads to the discovery of diabetes. Blood sugar estimations 1–2 hours after a carbohydrate-rich breakfast, repeated every year, in all direct relatives of each diabetic would permit earlier recognition of the disease in persons predisposed. See p. 811 about the laboratory methods of investigation.

a) Renal Glycosuria

The *familial renal glycosuria* has a limited capacity of tubular reabsorption of glucose alone. The morphology of the proximal tubules seems unchanged, but single tubules may be shortened. Depending on their number, the renal threshold for glucose can be so much lowered that even during fasting, and with perfectly normal blood sugar, glycosuria is constantly present, or with the renal threshold less depressed, glycosuria is intermittent only. The Tm_{GI}, the maximum capacity for the reabsorption of glucose, is not always reduced, but the amount of filtrated glucose always bears a definite relation to the amount of the reabsorbed glucose (REUBI, 1959; FROESCH, 1957; KRANE, 1966).

The innocuous anomaly is probably inherited through an autosomal dominant gene, the mild cases are possibly heterozygous, and the severe cases homozygous (FROESCH, 1957). No connection with diabetes mellitus has been confirmed.

The renal glycosuria due to congenital or acquired damage of the proximal tubules, the morphology of which can be usually demonstrated (SWAN'S neck tubule), is to be differentiated from the familial glycosuria. In the former two conditions, phosphate, amino acids and bicarbonate, in addition to glucose, are inadequately reabsorbed (de Toni-Fanconi-Debré syndrome).

As a rule, osteomalacia resistant to vitamin D predominates in the clinical picture.

The disorder may be congenital and inherited through an autosomal recessive gene. It may develop from a congenital metabolic disorder (cystinosis) or, reversibly or irreversibly, through exogenous (malate, lysol, heavy metals) or endogenous (Bence-Jones (myelom) protein, nephrosis) noxious agents (LEAF, 1966).

The physiological increase in glomerular filtration during pregnancy can lead to a fall in the renal threshold for glucose, and thus to renal glycosuria. This *glycosuria of pregnancy in the presence of normoglycemia* is today considered diabetes-suspect, even when it disappears with the termination of the pregnancy.

Glycosuria of the newborn occurs in the majority of infants during the first few days or weeks of life. Prematurity prolongs its duration. It is better described as *neonatal mellituria*, since, besides glucose, lactose, fructose and galactose are also found in the urine. Functional immaturity of the renal tubules and of the liver presumably are the cause of the excretion of glucose and galactose. Increased reabsorption of lactose from the intestine explains the concomitant lactosuria.

b) Cyclic or Alimentary Glycosuria

The result of the oral glucose tolerance test is normal in *cyclic or alimentary glycosuria*. Intermittent glycosuria is restricted to postprandial periods, whereas urine voided during the fasting state is sugarfree. This may be renal glycosuria with a slight fall in the renal threshold, which has a good prognosis, or an early stage of true diabetes mellitus with lowered renal threshold. The prognosis of the latter is uncertain and is influenced by medical supervision.

Primary essential diabetes with an inherited predisposition, with abnormally high and prolonged hyperglycemia after carbohydrate intake associated with the excretion of pathological amounts of glucose in the urine is the predominant cause of glycosuria. *Secondary diabetes* can be due to pancreatic or extrapancreatic factors.

Insular-insufficiency diabetes develops following damage or loss of the B-cells of the islets. It is reversible in acute pancreatitis and in prolonged starvation, and irreversible in advanced fibrosis of the pancreas, in hemochromatosis, and after pancreatectomy. See p. 748, 808 for pharmacological and experimental pancreatic diabetes.

Extra-insular diabetes is due to the mobilization of glycogen under the influence of hormones or to increased gluconeogenesis or to diminished breakdown of glucose in the periphery. It is primarily reversible, only progressing to true, irreversible diabetes in the presence of a predisposition.

A large release of *adrenaline* can occur with any somatic or mental stress, and in particular with CLAUDE BERNARD'S "piqûre", with brain injuries, brain tumors, encephalitis, meningitis, injuries to the soft tissues, fractures, and myocardial infarctions. It is due to increased glycogenolysis in the liver with the simultaneous inhibition of the secretion of insulin. A *pheo-*

chromocytoma which is active for a long time can lead first to intermittent and later to permanent diabetes.

Glycosuria is very rarely due to a *tumor of the A-islet cells* producing glucagon (see p. 823).

In *acromegaly*, the growth hormone probably leads to poor utilization of glucose in the periphery through the release of free fatty acids, if sufficient additional insulin cannot be liberated (see p. 113). See p. 86 for idio- and meta-pituitary experimental diabetes.

In *Cushings's syndrome* or during treatment with glucocorticoids (cortisone, prednisone), gluconeogenesis in the liver is increased (See p. 303, 351).

The reduced glucose tolerance of *primary aldosteronism* is rarely associated with glycosuria, and is due to potassium deficiency, which impairs the formation of glycogen and, above all, delays the release of insulin. We do not know whether *hyperthyroidism* can lead to clinical diabetes alone, or only when associated with potential diabetes.

In *oxi-hyperglycemic glycosuria* (LAWRENCE) [tachyalimentation (WILLIAMS), pseudodiabetes] the blood sugar rises quickly to high values after ingestion of food or glucose, on account of an accelerated absorption of carbohydrates, and then falls rapidly to normal or subnormal levels. This reaction is most striking in the oral glucose-tolerance test. Temporary glycosuria appears, and hypoglycemic symptoms are common. It occurs after gastroenterostomy or gastrectomy with the consequent accelerated emptying of the stomach, in hyperthyroidism and in vegetative dystonia. The intravenous tolerance test and the tolbutamide test yield normal results. Alimentary glycosuria may result from this disorder.

Similar oral glucose loading curves are met in the rare cases of glycosuria due to delayed or impaired deposition of glycogen. This is found only in combination with one form of *glycogen-storage disease* (Type 1, von GIERKE, glucose-6-phosphate deficiency) and also, very rarely indeed, with severe *liver dystrophia* (so-called hepatic glycosuria).

Though *glycuria* is infrequent, its diagnosis is of importance to avoid treatment of an incorrectly diagnosed diabetes and because the recognition of fructose or lactose intolerance or of galactosemia is of vital significance.

Every case of mellituria with normal or subnormal glucose tolerance tests must be further investigated, and the sugar or sugar-like substance in the urine must be identified. This can be done most simply with enzymatic methods or by paper chromatography (CONSTAM, 1957; CONSTAM and ISLER, 1957).

Pentosuria. Alimentary pentosuria, i.e. the excretion of arabinose or xylose, can occur intermittently in normal and in diabetic individuals after the intake of food rich in pentose. On the other hand, in *essential pentosuria*, *xylulose* (xyloketose) is continually lost in the urine, regardless of food intake. This anomaly is inherited on an autosomal, recessive gene and is due to defective TPN-xylite-dehydrogenase, an enzyme which activates the conversion of xylulose into xylite (HIATT, 1966). Both forms of pentosuria can occur alone or in combination with diabetes mellitus.

Fructosuria, levulosuria, is observed in normal persons after excessive intake of fructose. Such *alimentary fructosuria* is rare, since the limit of assimilation for this sugar is seldom exceeded. *Essential fructosuria* is the result of a deficiency of fructokinase. Apart from the polyuria following the intake of large amounts of fructose, these patients have no other symptoms. Their life expectancy is normal. Intake of fructose causes an abnormal rise of fructose in the blood and a reduction of blood glucose. *Fructose intolerance*, another disorder of the metabolism of fructose, is inherited on an autosomal, recessive gene. It is caused by a deficiency of the fructose-1-phosphate-aldolase and may lead to severe hypoglycemia, liver damage, and retarded growth. The diagnosis is made by carefully injecting fructose intravenously, which results in hypoglycemia and hypophosphatemia (FROESCH, 1966).

Galactosuria and galactosemia. Hereditary congenital galactosemia must be differentiated from the mild *physiological galactosuria* in normal babies and from the *secondary galactosuria* in liver diseases. Whereas amino acids and protein are excreted in the congenital type, lactose or glucose or even fructose sometimes are found in the urine in the other two conditions.

Galactosemia is a metabolic disorder, inherited on an autosomal recessive gene. The body is unable to convert a sufficient amount of galactose into glucose, due to a deficiency of galactose-1-phosphate-uridyltransferase. The toxic intermediate metabolic products lead to malnutrition, hepatic cirrhosis, cataract, debility and death. The diagnosis can be made by showing the absence of this ferment in the erythrocytes of these patients, even in the blood obtained from the umbilical cord. The damage is reversible, provided a galactose-free diet is instituted at the right time (INSELBACHER, 1966).

Lactosuria is met in normal women about 6 weeks before delivery, and persists until after lactation. It is also observed in healthy infants during the period of breast feeding. In both cases, galactosuria is an accompanying feature;

there are no subjective symptoms and treatment is not necessary.

The *lactosuria of lactation* must be differentiated from the lactosuria due to *lactose intolerance*, which causes diarrhea and occasionally is accompanied by lactosuria, glycosuria and intestinal malabsorption. Treatment involves the elimination or the reduction of lactose in the diet.

Saccharosuria results from a disturbance of one or several of the disaccharidases. It leads to an accumulation of saccharose in the intestine, to fermentation and diarrhea. Occasionally this may lead to the absorption of disaccharides and to their excretion in the urine. Here as well, all food containing saccharose must be avoided.

Mannoheptulosuria follows the intake of large amounts of certain Avocado pears. D-Mannoheptulose is excreted in the urine. The condition has no clinical importance, unless it is mistaken for diabetes.

5. Prognosis

As statistics from the Joslin Clinic in 1957 showed, before the discovery of insulin, six years was the average duration of diabetes from its diagnosis till death. In 1956/57 it had increased threefold and the present day figures are even more favorable. This is the result of insulin therapy particularly, but also of the modern improved methods for the treatment of infections and circulatory disturbances, earlier diagnosis, and better training of physicians and patients. The value of the oral hypoglycemic tablets cannot yet be definitely appraised; probably they are of importance too.

ROOT estimated the life expectancy of the diabetic to be at least 3/4 of the normal expectancy. Among 44000 diabetics, he found about 3000 had survived the average life expectancy reckoned from the time the first diabetic symptoms appeared.

According to the figures of WHO, the mortality due to diabetes in Germany has increased from 10.2 in 1950/52 to 13.6/100000 inhabitants in 1960/61. In Switzerland, these figures have risen from 13.4 to 14.7, and in U.S.A. from 16.3 to 16.7 per 100000 inhabitants.

As Table 6a indicates, *ketoacidosis* was the most common cause of death in diabetics before 1922, i.e. before the introduction of insulin. Today, *renal, cardiac and circulatory disorders* have taken over this position. The cases of death resulting from *infections*, in particular *tuberculosis*, are also on the decline, thanks to the combination of modern chemotherapeutic agents and antibiotics with insulin therapy. The increase of *cancer* among diabetics can be explained by the longer life expectancy of diabetics and by the improved diagnostic methods. Comparable investigations in the same age groups suggest that, with the exception of *carcinoma of the pancreas*, malignant tumors do not occur more frequently in diabetics than in the metabolically healthy population. The latter, according to OAKLEY and co-workers, has a frequency of 4% of all the carcinomas in the general population, amongst diabetics 14%. WARREN *et al.* reported in 1966 10.7% carcinoma of the endometrium amongst 214 autopsied diabetic women dying from cancer, compared with 5% in nondiabetic female patients. According to MEISSNER and LEGG and to MOSS, endometrial cancer is twice as common in diabetics. So far, we have not noticed a preponderance of this localization of carcinoma in diabetic women. *Hypoglycemia* as a cause of death can be avoided by dividing the daily insulin requirement into several smaller doses.

In infantile and juvenile diabetes, *ketoacidosis* is still a common cause of death. KRAINICH and STRUWE found this in 3/4 of their cases, and the Joslin Clinic in 44%. The majority of the patients who developed diabetes before the age of 20 and subsequently lived

Table 6a. Principle causes of death of diabetics of the Joslin Clinic[a]

Causes of death	1898 to 5. 31. 14 (%)	6. 1. 14 to 8. 6. 22 (%)	1. 1. 50 to 12. 31. 55 (%)	1. 1. 56 to 12. 11. 57 (%)
Diabetic coma (primary)	63.8	41.5	1.3	1.1
Cardiovascular, renal	17.5	24.6	76.3	77.7
Infections other than tb	7.4	12.7	5.4	3.9
Tuberculosis	4.9	4.9	0.7	0.2
Cancer	1.5	3.8	10.3	11.3
Accidents	0	0.8	2.0	1.7
Inanition	0.3	2.2	0	0
Suicide	0.3	0.2	0.5	0.5
Hypoglycemia	0	0	0.2	0.2
Other and unknown	4.3	9.3	3.3	3.4

[a] From JOSLIN *et al.*: The treatment of diabetes mellitus, 10th ed. Philadelphia: Lea & Febiger 1959.

for over 10 years died from *nephropathy. Coronary* and *cerebral circulatory disorders* were the causes of death in those developing diabetes later in life. Our own series of observations on patients who survived the first clinical manifestation of their diabetes for 20 years or more show that 48% died from myocardial infarction, 16% from cerebral ischemia and 16% from uremia. As already mentioned, the prognosis of diabetes is greatly influenced by the renal, cardiac and circulatory disturbances. These may be accompanied by changes in the nervous system, and are collectively termed the "*diabetic angio-neuropathy*" (see Chap. 9).

Investigations on patients observed for more than 20 years show that the severity of the metabolic disorder has little influence on the prognosis, providing ketoacidosis can be avoided, but that the late symptoms grow in severity and frequency with advancing age of the patient and duration of diabetes. Poor treatment promotes the development of diabetic angio-neuropathy, whereas correct treatment has the opposite effect (Table 7). The quality of control of diabetes during the first 5–10 years seems of particular importance for the long-term prognosis (CONSTAM, 1965, 1970; CAIRD, 1969). Early and good treatment gives to a diabetic the best chances for protection from vascular complications for 30 or even 35 years. He can preserve his working capacity for more than 40 years by keeping angio-neuropathy at bay.

Table 7. Relation between the quality of treatment and the further course of diabetes

	Quality of treatment								
	Always poor			Variable			Always good		
	Duration of diabetes in years								
	20	25	30	20	25	30	20	25	30
No late symptoms	3	2	2	31	6	2	74	39	14
1–2 late symptoms	10	4	0	83	37	15	33	29	19
More than 2 late symptoms	67	22	6	126	88	48	13	12	10
Total number of diabetics	80	28	8	240	131	65	120	80	43

There are rare cases of diabetes, less than 3% of our patients, who, in spite of poor control, remain free from renal or circulatory disturbances. They present a special, not yet well defined type of diabetes.

The working capacity, the periods of absence from work, and the efficiency of the diabetic are dependent on the age of the patient, the duration of diabetes, the quality of treatment, and the time at which an effective therapy was started. Intelligence and energy are indispensable to keep a diabetes well controlled, and for the very same reason well stabilized diabetics have proved to be excellent workers in various professions and arts. On the other hand, poorly regulated patients are irregular also in their professional performances and often compelled to interrupt their work.

Blindness, nephropathy with edema and hypertension with cardiac failure make young diabetics unfit for work. Gangrene, cardiac insufficiency, or cerebral palsy, and less often, neuropathy cause *incapacity for work* in the older diabetics.

6. Therapy

a) Aim and Principles of the Treatment of Diabetes

Treatment should not only aim at prolonging life, but at obtaining normal growth and development, normal efficiency and life expectancy, and physical as well as psychic well being of the patient.

The earlier treatment is started, the simpler and the better its long-term prognosis. Therefore, we should do our best to recognize the disease as early as possible and to arrest its further evolution, i.e. to prevent potential diabetes from becoming latent, latent from developing into clinical and clinical diabetes from progressing to complete insular insufficiency.

We try to achieve this by *reducing the endogenous insulin requirement.* If this is not sufficient, we may, in addition, *accelerate the release of endogenous insulin* or slow the *intestinal absorption of carbohydrates and increase the peripheral action of insulin* or *substitute exogenous insulin* for the endogenous hormone.

Complete control, i.e. normal blood sugar values at all hours of the day, is possible only in early recognized cases. An effort should be made to attain the blood sugar values presented in Table 8. The sugar in the urine should disappear whenever possible, and, in severe cases, should not exceed 10–20 g/24 hours. A glycosuria of even 30 g/day must be accepted in individual cases of labile diabetes, if severe hypoglycemia is to be avoided. Polyuria and acetonuria must be suppressed at all costs.

In exceptional cases, we are sometimes compelled to deviate from these rules, such as in professions with irregular meal times, patients with mental disease and in persons unwilling to follow the treatment. The "free normal diet" is the choice left open where there is a lack of intelligence, and also for children who are

Table 8. Guiding principles for "good" and "fair" control

Overt diabetes	Blood sugar in mg/100 ml			Glycosuria per 24 hours	Acetonuria
	Fasting	1 hour after breakfast or lunch	2 hours after breakfast or lunch		
With diet alone	< 1.20	< 1.60	< 1.30	0	0
Diet + tablets	< 1.20	< 1.80	< 1.30	0	0
Diet + insulin, good	< 1.20		< 1.50	< 5% of carbohydrate intake	0
Diet + insulin, fair	< 1.30		< 1.80	< 10% of carbohydrate intake	0

inadequately supervised at home. Treatment with insulin is then restricted simply to preventing ketonuria and loss of weight. The long-term prognosis in most of these cases is, however, unfavorable.

Drastic measures should be avoided in patients older than 65 years, except when there is a vital indication, such as an incipient coma, an unavoidable operation or a complication. When an overweight patient of more than 70 years has suffered from diabetes for more than 10 years and this caused no complaints nor late symptoms, this proves that the patient has adjusted his mode of living well to his illness, and it is advisable not to change the situation, at least not in a radical way. As long as there is no ketonuria, high blood sugar values of up to 260 mg/100 ml after meals are of little significance in the cases already in existence for a long time and without complications or complaints, provided glycosuria is relatively moderate.

The treatment must be applied constantly by the patient or his relatives. In addition to treating and supervising the patient, educating the diabetic is an important part of the treatment and, therefore, the duty of the physician. A severe psycho-organic syndrome, indifference, debility, or hypochondria can render education impossible, but it is indispensable for the great majority of diabetic patients.

The success of the treatment should not be based on the laboratory findings alone. The subjective symptoms of the patient, particularly his professional efficiency, are as important as the objective results of the medical examination.

b) General and Dietary Treatment

Treatment with diet, muscular activity, avoidance of infections and mental stress all serve to reduce the endogenous insulin requirement.

The *method of diet prescription* should be simple and adequate for use in infantile, juvenile and adult diabetics, for overweight, normal and underweight patients, and even for cases with labile diabetes.(Table 9).

Diet prescriptions are necessary (Table 10 gives an example) even in the stage of potential, and even more in that of latent diabetes, to protect the predisposed islet cells from overwork.

Table 9. Seven fundamentals for permanent dietary treatment

1. The *diet prescription* must be given in writing, contain not only what is forbidden, but also what is permitted, not only the daily amount of food, but also its distribution amongst the various meals of the day.
2. Adaptation of the diet as much as possible to the individual needs and possibilities.
3. No starvation diet!
4. Distribution into as many single meals as possible.
5. Carbohydrates are the main producers of sugar, fats the main suppliers of calories, proteins are indispensable for the formation of tissues and enzymes; they are partly converted into carbohydrates and fats.
6. Restrict the insulin requirement by avoiding "surplus caloric import" (exceptions; children and people over 65 years of age).
7. Prevent the rapid absorption of carbohydrates by eliminating food with highly concentrated carbohydrates, and by combining carbohydrates with proteins and/or fats in the same meal.

Table 10. Diet prescription for potential and latent diabetes

Avoid completely:

Sugar, food or drinks sweetened with sugar, beer, honey, liqueurs, malt-containing food such as ovaltine, syrup, sweet cider, sweet wines, pastry.

Use in moderate amounts and per meal only one of the following items:

Cereal, bread, potatoes, oats, barley, rice, corn, millet, flour, noodles, spaghettis.

Overweight persons have to omit also:

Butter, fat, oil, cream, egg yolk, bacon, nuts.

Between the 3 main meals (breakfast, lunch, supper) and before retiring, 3 snacks consisting of bread or fruit or milk are recommended.

The so-called "qualitative diet", in which only the food richest in carbohydrates is quantitatively fixed, usually is adequate for diabetics who are controlled by diet alone without the aid of hypoglycemic drugs (Table 11).

Table 11. "Qualitative diet" for diabetics controlled without hypoglycemic drugs

Fats in particular (butter, oil, cream, egg yolk, nuts) must be restricted or omitted for overweight patients, skimmed milk substituted for whole milk, lean cheese for cream cheese, lean meat for fat meat, salads made with vinegar or lemon juice, meat grilled or boiled, not roasted, sausages are to be avoided.

To be avoided completely:

Sugar, sweetened foods and drinks, beer, honey, liqueurs, ovaltine etc., syrups, apple juice, sweet wines, cakes, pastries, bananas, dates, figs, raisins, grapes, currants

Permitted without restrictions:

to sweeten:
Assugrin, disorb, saccharine, sionon, sucaryl

to season:
Vinegar, pepper, salt and other spices

Drinks:
Water, beef tea, meat broth, coffee, tea, mineral water without additional substances, completely fermented wines

Vegetables containing few carbohydrates:

Red cabbage, endives, cucumber, lettuce, cress, dandelion, silver beet, mushrooms, rhubarb, pickled cabbage, celery stalk and leaves, asparagus, spinach

The following recommended *distribution of the food* should be changed only with the permission of the physician.

1. *Breakfast:*	White bread ... grams, or dark bread ... grams Milk or yoghurt Butter Cheese Coffee or tea
2. *Mid-morning snack:*	Bread[a] Butter[a] Cheese Fruit (exceptions given above)
3. *Lunch:*	Vegetables (in particular those listed above) White bread ... grams, or dark bread ... grams, or ... medium size potatoes, or ... ounces of barley, or oats, or millet, or maize, or ... drachms of rice Meat, or fish, or sausages, or eggs, or cheese Fruit (exceptions given above) Butter, fat, oil, cream
4. *Tea:*	Like 1st or 2nd meal
5. *Dinner:*	Like lunch[a] Milk[a]
6. *Before retiring:*	Like mid-morning snack

[a] Cross out if not applicable, ... physician must fill in corresponding amounts. 20 g brown bread = 16 g white bread = 1 medium sized potato = 1 table spoon of oats, barley, millet, maize = 1 dessert spoon rice or noodles or fluor.

Reduction in weight is more essential than the limitation of carbohydrate intake in the overweight diabetic patient.

The diet poor in fat and limited in carbohydrates is nearly always successful. The patients do not feel hungry if their food intake is distributed amongst 5–7 meals a day (Table 11).

Patients who need drugs to lower the blood sugar, tablets or insulin, should eat about the same amount of blood sugar-forming food every day at about the same time. Otherwise, the hypoglycemic agents will have one day too much, another day too little action.

To obtain the necessary stability of the diet, foods are grouped as shown in Table 12, and *food equivalents* (Table 13) permit patients a satisfactory variation in their diet besides the uniformity in its composition.

Some of the foods rich in carbohydrates also contain proteins and fats, but only in relatively small amounts. In cases where the protein content cannot be neglected, glucose formation from proteins is considered in our exchange tables. Under *free foods* are listed items which either contain very little carbohydrates or are normally consumed in such small amounts that they have practically no influence on blood sugar.

In Germany and Austria, the carbohydrates are usually measured in *bread units*, one unit corresponding to 12 g of carbohydrates. The term *bread equivalent*, preponderantly used in Switzerland, implies the amount of bread, cereal, oats, barley, potato, rice which supply 10 g of carbohydrates, inclusive of the glucoplastic amino acids. One *vegetable equivalent* represents the corresponding amount of vegetables, one *fruit equivalent* that of fruit or berries. Bread-, vegetable- and fruit equivalents contain equal amounts of carbohydrates, but they are physiologically different. According to JOSLIN, from bread the sugar rushes into the blood, from fruit it flows and from vegetables it seeps.

One *milk equivalent* corresponds to the quantity of milk or milk products, with the exception of cream, which yields about 10 g of carbohydrates and 7 g of protein. A *protein equivalent* represents the amount of meat, fish etc. containing 10 g of proteins, a *fat equivalent* the portion of butter, fat, oil etc., which forms 10 g of fat, whereas a *cream-nut equivalent* furnishes 10 g of fat, 1–6 g of carbohydrates and 2–6 g of proteins.

The corresponding values of the usual food stuffs are presented in Table 13.

In practice, the dietary conditions are most simply arrived at by giving a basic diet to begin with, watching its effects, and adding corrections subsequently if necessary.

Table 12. Classification of foods according to their main nutritional content

1. Carbohydrates		2. Proteins	3. Fats	4. Free foods of unimportant nutritional value
a) *Bread equivalent*	*Milk equivalent*	*Protein equivalent*	a) *Fat equivalent*	a) Baking powder, meat, broth, coffee, tea, curry, pepper, salt, vinegar; synthetic sweetening substances
b) *Fruit equivalent*		Meat	b) *Cream* and *nut equivalent*	b) Drinks containing no carbohydrates
c) *Vegetable equivalent*		Eggs		
		Cheese		
		Poultry		
		Venison		

Table 13. List of "exchangeable" foods. (Taken from G. R. CONSTAM: List of foods for diabetics)

a) Cereals and flour potatoes rice carbohydrate rich vegetables

1 German bread unit corresponds to		1 Swiss bread equivalent corresponds to
15 g	Crackers	12 g
21 g	Rolls	16 g
21 g	Peas, green; pea-flour	16 g
15 g	Barley, grain	12 g
12 g	Barley, flour	10 g
15 g	Gruel from wheat or maize	12 g
18 g	Oats, flakes, porridge, flour	15 g
18 g	Millet	15 g
60 g	Potatoes, fresh, cooked or uncooked	50 g
40 g	Potatoes, fried	35 g
15 g	Potatoes, baked	12 g
40 g	Chestnuts, fresh with shells	35 g
35 g	Chestnuts, shelled	30 g
20 g	Lentils, uncooked	16 g
60 g	Lentils, cooked	50 g
18 g	Maize, grains	15 g
12 g	Corn/maize, flour; maize-meal	10 g
12 g	Rice, or rice flour	10 g
25 g	Rye bread	20 g
25 g	Brown bread	20 g
40 g	Soja flour, with oil removed	35 g
12 g	Noodles, macaroni etc.	10 g
25 g	Whole wheat bread	20 g
21 g	White bread	16 g
16 g	Rusks sugarless	13 g

b) Vegetables

1 German bread unit corresponds to		1 Swiss vegetable equivalent corresponds to
*	Red cabbage, cooked	*
*	Cauliflower	*
200 g	Beans, green, young fresh, uncooked	175 g
25 g	Beans, baked	20 g
170 g	Beans, pods with large seeds	140 g
*	Water cress	*
*	Chicorée	*
*	Champignons	*
*	Endivie	*
85 g	Peas, green, fresh, shelled, uncooked	70 g
120 g	Peas, green, fresh, shelled, cooked	100 g
150 g	Fennel, uncooked	120 g
*	Cucumber	*
240 g	Cabbage, uncooked	200 g
100 g	Carrots, uncooked	80 g
150 g	Carrots, cooked	125 g
210 g	Turnips, uncooked	200 g
*	Cress	*
200 g	Leek, uncooked	175 g
180 g	Dandelion	170 g
*	Silver beet, leaves	*
240 g	Red peppers, uncooked	200 g
*	Mushrooms	*
240 g	Radishes	200 g
*	Rhubarb	*
170 g	Brussel's sprout	140 g
180 g	Beet root, cooked	150 g
*	Green salad	*
*	Pickled cabbage	*
120 g	Viper's grass, uncooked	100 g
240 g	Celery bulb, uncooked	200 g
*	Asparagus	*
*	Spinach	*
240 g	Tomatoes	200 g
*	Savoy cabbage, cooked	*
130 g	Onions, uncooked	110 g

c) Fruit and berries

1 German bread unit corresponds to		1 Swiss fruit equivalent corresponds to
100 g	Apple, fresh	80 g
22 g	Apple, baked	18 g
120 g	Oranges, peeled	100 g
120 g	Apricots, with seeds, fresh	100 g
18 g	Apricots, dried	15 g
90 g	Bananas, fresh, with skin	70 g
75 g	Pears, fresh	60 g
150 g	Blackberries	125 g
150 g	Strawberries, garden	125 g
240 g	Strawberries, wild	200 g
260 g	Grapefruit, with peel	220 g
240 g	Bilberries	200 g
170 g	Raspberries	140 g
200 g	Red currants	170 g
75 g	Cherries, sweet, with stones	60 g
200 g	Mandarines, with peel	170 g
200 g	Melon, with peel	170 g
120 g	Peaches, with seed	100 g
110 g	Plums, with stones	100 g
300 g	Lemons, with peel	250 g

d) Milk products poor in proteins and fats

1 German bread unit corresponds to		1 Swiss milk equivalent corresponds to
240 g	Butter milk	200 cc
200 g	Yoghurt	160 cc

Table 13 (Continued)

1 German bread unit corresponds to		1 Swiss milk equivalent corresponds to
400 g	Kefir	330 cc
240 g	Skimmed milk	200 cc
240 g	Whey	200 cc
300 cc	Curdled milk	250 cc
240 g	Full milk	200 cc

e) Proteins

	1 protein equivalent corresponds to
Meat, uncooked	50 g
Meat, cooked	40 g
Brain	100 g
Tripe	50 g
Liver, cooked	40 g
Smoked meat	40 g
Ham, uncooked	60 g
Ham, cooked, smoked, salted	40 g
Tongue	50 g
Sausages of different types av. erage	60 g
Fish, without skin and bones, raw	60 g
Fish, without skin and bones, cooked	50 g
Eggs, hens', without shell	80 g
Cheese lean	35 g
Cheese, at	40 g

f) Fats

	1 fat equivalent corresponds to
Butter, fresh	12 g
Egg yolk	20 g
Bone marrow	12 g
Margarine	10 g
Olive oil	10 g
Lard	10 g
Bacon, smoked or salted	15 g
Bacon, cooked	20 g

Table 14 gives an example of a basic diet for an adult with sedentary occupation. The figures in small print below the carbohydrate, protein, fat and calories represent average values for 1 equivalent in order to permit an approximate estimation of the composition of the diet.

Patients who cannot be trusted with the simple multiplication in such a diet, can see from Table 15 how many grams of apple or orange etc. are allowed at a specific meal.

If the patient continues to excrete sugar despite the basic diet, the bread at lunch and dinner can be omitted. If this is not sufficient, then stronger hypoglycemic agents are needed. If, however, the blood sugar values have become almost normal, the diet may be extended to include more bread equivalents at lunch and dinner, and more fruit equivalents before retiring etc.

For adults performing heavy work, the bread equivalents of all meals have to be increased. If breakfast is very early, two mid-morning snacks may become necessary. Diabetics who must lunch at or near work need at least 4 bread equivalents for this meal, giving them the possibility of eating weighed sandwiches brought from home, in addition to their fruit equivalents.

The weighing of food appears cumbersome at the start. However, after some experience, this does not cause any considerable delay in preparing meals. Observant diabetics can develop the ability to estimate the amount of vegetables, fruit and meat correctly within a few weeks. It is important that the carbohydrate-rich bread equivalents are always correct. When eating in a restaurant or while travelling, or when not at home, the bread equivalents should be

Table 14. Average basic diet for an adult with sedentary occupation, under treatment with hypoglycemic agents

	Breakfast	Mid-morning snack	Lunch	Tea	Dinner	Before retiring	Total	CHO	Protein	Fats	Calories
Bread equivalent	3		1	3	1		8	80^{10}	16^{2}	8^{1}	456^{57}
Vegetable equivalent			1[a]		1[a]		2	20^{10}	2^{1}		88^{44}
Fruit equivalent		2	2			2	6	60^{10}	6^{1}		264^{44}
Milk equivalent	1				1		2	20^{10}	14^{7}	16^{8}	280^{190}
Protein equivalent	1		2		2		5		50^{10}	30^{6}	470^{94}
Fat equivalent	1		1	1	1		4			40^{10}	360^{90}
Cream-nut equivalent											
							Total	180	88	94	1918

[a] Maddition, vegetables marked with * in Table 13 are free to be used or not.

chosen in the form of bread or potatoes, because their quantity can easily be estimated. This also applies to fruit, but farinaceous foods and stewed fruits must be avoided, as must soups and gravy, since their flour content cannot be recognized.

Table 15. Quantitative diet prescription for diabetics of below average intelligence

1. *Breakfast:*	Dark bread ... g, or white bread ... g, (20) (16) or oat meal ... tablespoons (1) Milk ... cc, or yoghurt ... cc (2) (1.6) Coffee or tea without sugar as desired Butter[a] ... Cheese ... g, or eggs ... g, or meat ... g (40) (80) (40)
2. *Mid-morning snack:*	[b] Dark bread ... g, or white bread ... g (20) (16) [b] Apple ... g, or pear ... g, (80) (60) or tangerine ... g, or orange ... g (150) (100)
3. *Lunch:*	Clear meat broth, unlimited Brown bread ... g, or white bread ... g, (20) (16) or potatoes ... g, or oat meal ..., (50) (1) or barley ..., or corn ... tablespoons, (1) (1) or rice ... teaspoons (2) (all cereals must be weighed before cooking). Vegetables unrestricted (red cabbage, cauliflower, endives, cucumber, lettuce, water cress, dandelion, silver beet, mushrooms, rhubarb, salads, pickled clover and cabbage, celery leaves, celery stalks, asparagus, spinach) Beans, green ... g, or carrots ... g, (175) (80) or beet root ... g, or Brussel's sprouts . (100) (140) or celery ... g, or tomatoes ... g (200) (200) Meat ... g, or fish ... g, or sausages ... g, (50) (60) (70) or salami ... g, or smoked meat ... g, (40) (40) or eggs ... g, or cheese ... g (80) (40) Apple ... g, or pear ... g, or mandarine ... g, (80) (60) (150) or orange ... g, or peaches ... g, (100) (100) or apricots ... g, or strawberries ... g, (100) (125) or grapefruit ... g, or raspberries ... g (220) (140) Butter, fat, oil[a] ...
4. *Afternoon tea:*	like meal 1 or 2
5. *Dinner:*	like lunch, in addition milk[b] ... cc, or yoghurt[b] ... cc

[a] Physician must indicate whether none, little, much, unrestricted.
[b] Physician must cross out the inappropriate.

c) Treatment with Antidiabetic Tablets (Oral Hypoglycemic Agents)

Two groups of drugs – the sulfonylurea derivatives including pyrimidine and biguanides are extensively used today.

Table 16 gives a survey of the sulfonylurea and pyrimidine derivatives in use.

All the sulfonylurea derivatives act in the same way. They differ only in their toxicities, their potency per gram of substance, the duration of action and their side effects.

The main action of the sulfonylurea drugs is to stimulate the release of insulin from the

Table 16. The most commonly used sulfonylurea and pyrimidine derivatives

Carbutamide	Alantin Antidiabeticum Bucrol Butasulfe Glucidoral Invenol Midosal Nadisan Norboral Retarden Sulfadiabet
Tolbutamide	Artosin Dolipol Orinase Rastinon
Chlorpropamide	Catanil Chloronase Diabinese Diabetoral Mellinese
Glybenclamide, glyburide	Daonil Diabeta Euglucon
Tolazamide	Diabetas Tolinase Tolisan
Acetohexamide	Dinetin Dimelor Ordimel
Glycodiacine	Gondafon Lycanol Redul

B-cells of the pancreas. In nondiabetics, the release of insulin is primarily regulated by the glucose content of the arterial blood in the pancreas. In the diabetic, however, the damaged B-cells can no longer react to the hexose stimulus or the reaction is delayed. Sulfonylurea derivatives can mobilize insulin reserves still present. The insulin in the B-cells may be bound to zinc, in the form of large conglomerations of insulin molecules. The splitting up of these molecular complexes by sulfonylurea promotes the liberation of insulin from the B-cells. The insulin thus liberated, acts primarily on the liver, where it inhibits the release of glucose. The sulfonylurea compounds also potentiate the action of small amounts of insulin even in the complete absence of the pancreas. In dogs fed on a carbohydrate-free diet, they increase the capacity of the B-cells to produce insulin. Whether this holds true for man is not certain. Tolbutamide can release insulin from the insulin-antibody bondage in vitro and can also prevent insulin from combining with antibodies. Sulfonylurea derivatives also have an antithyroid effect, and some of them are bacteriostatic, although this is of no practical importance. The temporary inhibition of individual hepatic enzymes is just as unimportant as the increased excretion of endogenous citric acid when large doses of tolbutamide are used.

Side effects are the more unpleasant, the longer the duration of action of the drugs. When these drugs were introduced, side effects were observed in 5–11% of the cases treated with carbutamide, and in 1–3.2% treated with tolbutamide. They were more common with chlorpropamide, but only when the daily dosage exceeded 0.5 g. Today, side effects are considerably less frequent, due to the more precise indications for the use of these drugs.

The side effects caused by sulfonylurea derivatives may be toxic or allergic reactions of the skin, the bone marrow and the nervous system. They appear as *morbilliform rash* with high fever, as *urticaria*, as *photoallergic eczema*, as *exfoliative dermatitis*, as *thrombocytopenic purpura*, as *agranulocytosis* or as *hemolytic* or *aplastic anemia*. *Headache* not associated with hypoglycemia or dizziness or peripheral neuropathy, which is to be differentiated from diabetic neuropathy, may be symptoms of intolerance of the sufonylurea compounds. Occasionally, heartburn, nausea and abdominal pains may occur, due perhaps to the increased gastric secretion. Reactivation of *gastric or duodenal ulcers* causes the typical pattern of pain, even hemorrhage and perforation. A few patients complain of *constipation*. *Cholostatic jaundice* is especially common with overdoses of chlorpropamide. Several cases of *porphyria*, and several of *myocardial changes* have been reported. We have not so far observed *hypothyroidism*, which is thought to develop after years of treatment with sulfonylurea.

Incompatibility with alcohol, an antabuslike effect, considerably lowers tolerance to alcohol. The action of barbiturates is prolonged, whereas salicylates, sulphamethizole and phenylbutazone can potentiate the hypoglycemic power of the sulfonylurea drugs. PAS taken at the same time is said to produce headaches.

It is important to realize that the action of tolbutamide can be increased by the anticoagulant agents of the dicoumarol group.

Overdosage is dangerous and may lead even to fatal hypoglycemia. Even normal doses endanger patients with renal failure and delayed excretion of the drugs. Further, individuals who break down these drugs only very slowly can also be endangered (SCHULZ). The diagnosis of hypoglycemia is easily missed, its symptoms: weakness, dizziness, ataxia and confusion being mistaken for cerebral thrombosis. Secondary failure of the treatment, i.e. the sudden loss of hypoglycemic action after a period with satisfactory effectiveness, may lead to ketoacidosis in an unobservant patient. The right moment for the start of insulin therapy should not be missed.

Tables 17 and 18 summarize the indications and contraindications for sulfonylurea compounds. Table 19 is for the information of patients under this treatment.

Table 17. Indications for the use of sulfonylurea derivatives

1. Adults who have never had insulin, who cannot be controlled by diet alone and who have no signs of ketosis.
2. Adults in whom diabetes started after age 40 and was treated by insulin for less than 5 years and was controlled by 30 units per day or less.
3. Overweight patients may try oral treatment even after larger doses and longer use of insulin, but only after preliminary reduction of body weight.
4. Cases of insulin resistance justify a trial.
5. In latent diabetes, when the blood sugar 1 hour after breakfast is above 120 mg per cent, cautious oral treatment may be used to prevent further deterioration of carbohydrate tolerance.

Table 18. Contraindications to sulfonylurea therapy

1. Diabetes which can be controlled by diet alone.
2. Overt infantile or juvenile diabetes.
3. Insufficient action of sulfonylurea compounds.
4. Diabetic women who might become pregnant.
5. Ketoacidosis
6. Diabetics under stress.
7. Renal- or hepatic insufficiency.
8. Infected gangrene or progressive retinopathy.
9. Insufficient medical supervision.

Table 19. Advice for patients on sulfonylurea therapy

1. The effect of alcoholic beverages is markedly increased by these blood sugar-lowering agents.
2. Besides these tablets no other medication is allowed unless your doctor approves. Drugs for rheumatic pains or urinary infection are particularly likely to alter the potency of the blood sugar-lowering tablets.
3. Stop taking the tablets and notify your doctor immediately if one of the following symptoms appears:
   a) raised temperature;
   b) skin rash;
   c) severe headache;
   d) bleeding from nose, intestine, bladder, genital organs;
   e) incipient acidosis, i.e. nausea, and possibly vomiting, combined with much sugar in the urine.
4. *Take 1/2 tablet less:*
   a) in case of "insulin reaction" i.e. headache or trembling with sweating and weakness;
   b) prior to an unusual exercise like swimming, hiking, sport.
5. *Take 1/2 tablet more:*
   a) if your urine test shows sugar 2 days in succession;
   b) if after this the morning urine is not sugar free in 2 days, blood sugar determination is necessary.
6. Thirst, large volume of urine and strongly positive sugar tests demand immediate notification of the doctor, even on Sundays.

The choice of drug depends on the action of the drug and its duration of action. It is advisable to try tolbutamide first; it is the least potent, but also the least toxic. It must be given in two daily doses since its half-life is 8 hours. It is excreted by the organism in the oxidized form in the urine, precipitating as a whitish sediment on acidification, thus simulating albuminuria. If this preparation is not effective enough, one of the stronger preparations, carbutamide, glyburide or chlorpropamide, can be tried, and since they have half-lives of 40 and 34 hours respectively, they need only be given once daily. In our opinion, the other preparations have no great advantages over the four drugs mentioned above.

Without reduction of the needs for endogenous insulin at the same time, i.e. without dietary measures, the oral antidiabetic agents are inadequate and not effective for very long. *They should only be used in combination with diet.*

To stabilize diabetics who are not treated with insulin, 0.5 g of carbutamide or 5 mg of glybenclamide (glyburide), or 0.25 g of chlorpropamide are given with breakfast on the first morning. The blood sugar is measured and the leucocytes are counted in the late afternoon, as also on subsequent days. If the blood sugar measured in the capillary blood does not fall to below 140 mg%, then 1.0 g of carbutamide, or 10 mg of glyburide, or 0.5 g of chlorpropamide is given again on the following day. If the blood sugar is below 120 mg%, however, the dose on the following day is reduced by 0.5 g carbutamide or 2.5 mg of glyburide, or by 0.25 g of chlorpropamide. This procedure is repeated during the next few days. If the blood sugar falls, the carbutamide, glybenclamide or chlorpropamide dosage can be reduced. If a satisfactory fall of the blood sugar is obtained with 1 g of carbutamide or 5 mg of glybenclamide or 0.5 g of chlorpropamide, these drugs are replaced by the less toxic tolbutamide. The daily dose of this drug, however, must be greater (by 0.5 g) than that of the other 3 preparations and must be divided between a morning and an evening dose.

Continuation of the treatment with sulfonylurea drugs has little chance of lasting and favorable action if the blood sugar does not fall to below 120 mg% by the fifth afternoon.

When the leucocytes fall to below 3000 the treatment must be discontinued at once and the patient observed for the possible development of *agranulocytosis.*

The *maximum maintenance dose* per day, is 1.5 g of carbutamide, 15 mg of glybenclamide, 2.0 g of tolbutamide and 0.5 g of chlorpropamide.

The *transition from insulin to oral hypoglycemic therapy* can be made in stages or abruptly. In the latter case, the insulin is fully discontinued in the morning, and instead the patient is given 1.5 g of carbutamide or 10 mg of glybenclamide, or 0.5 g of chlorpropamide with breakfast. The blood sugar is checked by a quick method at 11 a.m., and if necessary, insulin can then be injected before lunch. If the blood sugar has still not fallen in the late afternoon, insulin therapy must be resumed and the trial with tablets abandoned. However, if the patient responds, i.e. if the true blood sugar is under 120 mg% between 5 and 6 p.m., the following day the doses of carbutamide or glybenclamide or chlorpropamide are reduced in the same way as described above, and tolbutamide eventually substituted for these long-acting preparations.

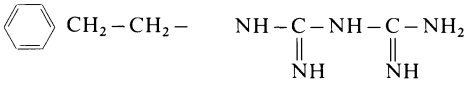
If diet and sulfonylurea derivatives are inadequate, their hypoglycemic action can be considerably enhanced by giving a biguanide in addition. The sulfonylurea treatment is continued, and biguanide is administered in the usual way, the dosage being slowly increased.

A trial of sulfonylurea preparations in combination with insulin is only worthwhile in certain rare cases of insulin resistance.

Table 20 gives a survey of the biguanides in use.

The actions of biguanides demonstrated in vitro on muscular and liver tissues are: increased uptake of glucose, enhanced formation

Table 20. Survey of biguanides currently used

Chemical term	Structural formula	Commercial name
DBI Phenylethylbiguanide		*Phenformin*, DBI, Debinyl
W 37 n-butylbiguanide	$CH_3-CH_2-CH_2-CH_2-$	*Silubin*, Silubin, Silubin retard
LA 6023 Dimethylbiguanide		HCl *Glucophage*, Metformin

of lactate, with reduced utilization of oxygen and diminished storage of glycogen, inhibition of various enzyme systems. However, we still do not know what effect these actions have in vivo.

In a test using labeled glucose, it was shown that biguanides also promote the breakdown of glucose in the periphery of healthy subjects, but causes gluconeogenesis at the same time in the liver, so that the glucose turnover rises, while the blood sugar remains constant. In the diabetic, the release of glucose from the liver is already increased and is little increased by biguanide, whereas the action in the peripheral breakdown is predominant and the blood sugar consequently falls (SEARLE, 1966). Probably the liver absorbs more lactate and reconverts it into sugar. The biguanides appear to act peripherally only on the muscle cells, lowering the threshold for the uptake of glucose at the cell membrane. They slow the absorption of carbohydrates from the intestine. The mode of action is still not fully explained. These compounds have no direct influence on the B-cells of the pancreas, and do not increase the plasma insulin, as do the sulfonylurea derivatives.

The side effects of biguanides may take the form of diarrhea, heartburn, loss of appetite, nausea, or vomiting. They may occur early and promptly disappear if the dose is reduced. Later, only 1–2 months after the start of this therapy, tiredness, weakness, and loss of weight occur. Some patients have a sensation of a metallic taste when drinking alcohol. Allergic skin changes are exceptionally rare and may present urticaria or pruritic erythema. In the medical literature, one case of thrombocytopenia has been attributed to this therapy.

The biguanides can also lead to hypoglycemia, which, however, is never severe. The development of *ketonuria* in the presence of normal or only slightly elevated blood sugar is dangerous and can be fatal. It must not be treated by intravenous administration of lactate, since the biguanides, at least phenformin, have the tendency to elevate the levels of lactate in the blood. A number of cases of severe *lactacidosis*, some with fatal outcome, have been described in the last few years. Diabetics with acute circulatory failure seem to be particularly prone, as are elderly people with renal insufficiency, and young diabetics with too little insulin. *In acute complications, the tablets must be replaced by insulin*, even when the blood sugar levels were normalized by biguanides.

Here, as well, there is the danger that the right moment for starting treatment with insulin may be missed. It is better in doubtful cases to start with insulin therapy, and then subsequently to try tablets.

Tables 21 and 22 summarize the indications or contraindications for using the biguanides.

Table 21. Indications for treatment with biguanide

1. Adult, overweight diabetics, in whom the diet alone is inadequate. Here we prefer biguanides to the sulfonylurea derivatives. If only a fair stabilization is obtained with the biguanides, a sulfonylurea preparation is added.
2. As an additional therapy in diabetics inadequately stabilized with sulfonylurea compounds.
3. In labile diabetes, where the stabilization with insulin and diet is unsatisfactory (trial).
4. In insulin resistance (trial).

Table 22. Contraindications to treatment with biguanide

1. Gastrointestinal disease.
2. Infected gangrene.
3. Circulatory, renal, or hepatic insufficiency.
4. Underweight.
5. Operations.
6. Women who may become pregnant.

We choose the weaker preparations, dimethyl- and butyl-biguanide in preference to phenformin, since we have never observed a case of acidosis with the former two compounds. Butyl-biguanide is also on the market as tablets with prolonged action.

During the first days, 1 tablet of 500 mg dimethyl-biguanide is given three times daily before meals, or 1 tablet of 100 mg butylbiguanide retard twice daily, in the morning and in the evening. If the desired decrease of the blood sugar is not obtained within 5 days, the dose is

increased by 1 tablet every fifth day, until the blood sugar is stabilized as required, or side effects appear and demand that the dose be reduced again.

Combined treatment with biguanides and sulfonylurea derivatives has been mentioned on p. 784.

d) Treatment with Insulin

Treatment with insulin is an imperfect substitution therapy, since the exogenous insulin injected subcutaneously, intramuscularly or intravenously, reaches the systemic circulation, whereas the endogenous insulin first passes from the pancreas into the liver by the portal vein, and only then reaches the general circulation. In addition, commercial insulin is probably changed during the process of extraction from the pancreas and therefore not identical with the normal hormone.

Treatment with insulin is necessary in the vast majority of cases of growth-onset diabetes. It is also indispensible in maturity-onset diabetes if diet, adaptation of the mode of living and possibly the use of oral hypoglycemic drugs are not adequate to control the disease. It is also indicated in the presence of severe late symptoms (such as progressive retinopathy, or infected gangrene, see p. 792/95), since, in addition to its hypoglycemic action, insulin also seems to have other effects (see p. 794). Insulin therapy is urgent in pre-coma and in diabetic coma. An early and rapid start of combined insulin and dietary therapy allows interruption of the administration of insulin for shorter or longer periods in some growth-onset diabetics.

Insulin therapy should start before the islet system fails completely, while reserves of endogenous insulin are still sufficient to control the postalimentary hyperglycemia after exogenous insulin has reduced the basic blood-sugar level. Insulin treatment leads to complete islet insufficiency, i.e. a form of labile diabetes with all the inconveniences this involves for the patient if it is started too late or inefficiently managed.

There are no absolute *contraindications* to treatment with insulin. Severe allergy to insulin must first be corrected. In elderly patients, the blood sugar must be reduced slowly. Mental deficiency or pronounced irresponsibility render the treatment insulin difficult, and may even make it impossible.

The ferments of the intestinal tract inactivate insulin so that it must be given by injection.

The action of insulin is prolonged by combination with proteins such as protamine or globin, or other complex substances, such as surfen, from which it is gradually liberated. The insulins of the *lente group* are not bound to other bodies; their prolonged action is achieved partly by their delayed solubility and partly by their crystallization. They represent suspensions of amorphous or crystalline insulin.

Table 23 gives a review of the most common types of insulin in use. Insulin is measured in *international units*. One unit is the amount of insulin which has the same action as 1/24 mg of a crystallized standard insulin.

The action of insulin varies with the individual. The *glucose equivalent*, i.e. the amount of carbohydrates utilized by 1 unit of administered insulin, varies according to the dose of insulin and to the blood sugar level or the time at which the injection was made. It is greater with small than with high doses. Massive single doses of insulin are considerably less effective per unit. In the presence of high blood sugar, the action per unit of insulin is delayed and smaller than in mild hyperglycemia.

Start of Insulin Therapy: Without diet, the effects of the treatment with insulin are unpredictable and irregular. Stabilization with

Table 23. The most commonly used types of insulin

Type	Appearance	Addition	Buffer	Possibility of mixing	Duration
Rapid					
Regular, soluble	Clear	None	None	With all other insulins	5– 7 hours
Actrapid	Clear	None	Acetate	Lente group	
Intermediate					
Globin	Clear	Globin	None	Regular	12–18 hours
Lente	Turbid	None	Acetate	Lente group	18–24 hours
NPH	Turbid	Protamine	Phosphate	Regular	18–24 hours
Semilente	Turbid	None	Acetate	Lente group	12–16 hours
Rapitard	Turbid	None	Acetate	Lente group	4–20 hours
Slow					
Protamine zinc	Turbid	Protamine	Phosphate	Regular	24–36 hours
Ultralente	Turbid	None	Acetate	Lente group	24–36 hours

insulin and diet should begin in a suitably equipped hospital.

The first dose is: in the adult 10 U; in children 4 U soluble insulin and is given 30–45 min before breakfast. This rule does not apply to cases of ketoacidosis and extreme underweight.

The following doses are based on the results of the urine tests before the three main meals, according to the "0–8 rule" (Table 24). The urine is examined with BENEDICT's qualitative reagent or with Clinitest-tablets. One hour before the test is to be performed, the bladder is completely voided and the urine discarded. The test is done on the "second" urine.

Table 24. "0–8 rule"

Benedict or clinitest	Acetone negative	Acetone positive	Repeat test after
Increase the dose by soluble insulin:			
Blue	0 U	0 U	4–5 hrs
Green-cloudy	2 (1)	4 (2)	4–5 hrs
Yellow-green	4 (2)	8 (4)	4–5 hrs
Brown	6 (3)	12 (6)	1 hour
Red or orange	8 (4)	16 (8)	1 hour

( ) = Dosage for children weighing less than 40 kg.
If food intake stopped (p. 788) repeat test after.

Example: A diabetic who has never received insulin, is given 10 U of soluble insulin before breakfast. The test for sugar in the urine before lunch is brown and acetone is negative. The patient then receives 6 U. The test before dinner is yellow/green and acetone-positive; the patient, therefore, receives 8 U insulin. On the following morning, the test is again yellow/green and acetone is positive. He then receives another 8 U in addition to the 10 U of the previous day, making a total of 18 U insulin. The urine test is blue at midday, so the dose of the previous day is given, i.e. 6 U. In the evening the test is brown again, and acetone is negative. The patient therefore receives 8 + 6 units = 14 U.

The blood sugar is measured at the latest on the third day, before lunch and supper, and before breakfast on the following morning. The insulin dosage can then be adjusted more exactly.

Ambulant adult diabetics, who are neither resistant to insulin nor ketotic should not be given more than 40 U at once. Children under 40 kg should not be given more than 24 U in one dose. After one to two weeks of intensive treatment with insulin there is usually some improvement in tolerance, so that the insulin dose can be decreased, and in certain cases, treatment with insulin may even be stopped. As soon as the insulin requirement has been constant for 2–3 days the physician can change from the rapidly acting soluble insulin to an insulin with prolonged action providing the following rules are adhered to:

In diabetics who need 10–30 U insulin per day a single injection of a "depot" insulin is usually adequate, the choice of preparation being dependent on the time of day at which the need for insulin is greatest.

Diabetics with a daily requirement of 30–50 U of insulin usually need 2 injections given at the same time, before breakfast. A rapidly acting insulin is given separately from a "depot" insulin.

If 50–80 U of insulin are needed per day, it is best to give two injections of an intermediary insulin, one before breakfast and the other before dinner in the evening.

The treatment of *labile diabetes*, or of patients who are not compensated with 80 U, necessitates the advice of a specialist in this field. The same applies to children, even if their insulin requirement is below 40 U daily.

Adjustment of the Dose of Insulin to Changes in Tolerance. We advise intelligent diabetics to increase the insulin dose by 2 U, if the urine voided before breakfast contains sugar on two consecutive days. This rule applies only to patients who are not bedridden, but are capable of normal activity. If bed rest is necessary, regardless of whether on account of illness or injury, soluble insulin is substituted for the long acting one. Half as much insulin as used the previous day is injected before breakfast, a little less than a quarter as much before lunch, and a little more than a quarter as much before dinner, if the urine sugar tests before the 3 main meals are negative. If a test is positive, the insulin dose is increased at once by 4 U in adults, by 2 U in children.

Hypoglycemic manifestations despite adherence to the prescribed diet and normal physical activity always require a reduction of the dose of insulin which caused the hypoglycemia. If this dose was less than 16 U it must be reduced by 2 U, if it was greater, by 4 U. If the hypoglycemia led to loss of consciousness the dose must be reduced by 8 U. Reactive hyperglycemia with glycosuria usually follows hypoglycemia. Therefore, the dose of insulin must be reduced after a hypoglycemic attack, even if glycosuria is present. The dose should be reduced by 4–6 U per half day *before* any unusual physical exertion; 6 U applies to strenous physical exertions such as field work, skiing or competitive sports.

Interrupted food intake due to lack of food or indigestion or loss of appetite should not

lead to discontinuation of insulin therapy, but to the application of "*rule 0–8*", since loss of appetite may be a sign of precoma. Table 24 mentions the insulin doses. Urine tests have to be repeated and the insulin injections, if necessary, renewed every 4–5 hours if the urine test is blue-yellowish-green, every hour if it is brown, orange or red.

Hunger in a diabetic indicates poor control with significant loss of sugar in the urine, or inadequate diet or too high a dose of insulin.

e) Side-Effects of Insulin

α) Hypoglycemia, Insulin Shock, Insulin Reaction (see also p. 783)

The development of hypoglycemic symptoms does not only depend on the concentration of the blood sugar, but rather on the rate at which the blood sugar falls, and, in addition, on the amount of glycogen still in storage. Therefore, patients can be found who feel well with a blood sugar of 30 mg% or less and others have symptoms even when the blood sugar is still elevated at 180 mg%.

Hypoglycemia is due either to *insufficient formation of blood sugar* or to excessive blood sugar consumption. The former is caused by poor food intake or indigestion, or, very rarely, by insufficient or non-mobilizable glycogen or fat reserves. *Increased blood sugar consumption* may be due to physical exertion, to overdosage of insulin or reduction of the insulin requirement, for instance after childbirth or surgical drainage of an abscess. Occasionally, improved tolerance is met without any recognizable reason, perhaps due to blocked insulin being liberated.

Diabetics with untreated pituitary or adrenal insufficiency, are very sensitive to insulin and predisposed to hypoglycemic shock. This applies also to diabetics who have lost weight, and those suffering from diarrhea.

The possibility of an insulin reaction must always be considered in patients treated with insulin who do not feel well, particularly when the symptoms arise shortly before meal time and improve with food intake. The typical *cardinal symptoms*, due in particular to adrenaline are: tremor, sweating, weakness and ravenous appetite. They may be absent especially when slow-acting insulin is used, in which case nervous symptoms such as headache, paresthesia in the oral region, and visual disturbances are prevalent. Some patients complain of pain in the pancreatic area. Silly, euphoric behavior is mistaken for drunkenness. A state of apathy or semiconsciousness and epileptic seizures terrify the patient's family. Cerebral disorders with paralysis and bilateral Babinski's sign may be difficult to differentiate from encephalorrhagia. Lasting cerebral damage, a psycho-organic syndrome, epilepsy and even death are possible.

The prognosis of a hypoglycemic shock depends on the dose and type of insulin, on the state of nutrition and the cardiovascular system, and above all on how fast appropriate treatment is started. Even hemiplegia with pseudo-bulbar palsy can disappear within a few hours or days. Tolerance of severe hypoglycemia by brain and heart is poor, and insulin therefore must be carefully handled. Particular caution demands the use of long-acting insulin, since hypoglycemic shock can appear very suddenly with no previous warning.

Hypoglycemia must be differentiated from diabetic coma, epilepsy, eclampsia, encephalorrhagia, alcoholic intoxication, poisoning, hyperventilation, tetany and psychoses; in children from acute infections beginning with convulsions. Hypoglycemic symptoms are sometimes similar to those of severe hyperglycemia, because both are due to the inadequate supply of sugar to the brain and muscle cells.

The differential diagnosis between insulin reaction and diabetic coma is presented in Table 25.

Prevention is based on regular food intake, correct in time and quantity, adaptation of insulin to changes in insulin requirement, for instance sport, or work, and appropriate schooling of the individual patient. The recognition of mild signs of hypoglycemia and the appropriate changes in the dosage of insulin are important.

Treatment: as longs as the patient can still swallow, he should immediately eat rapidly absorbable carbohydrates, such as sugar or bread, no fruit nor fructose. If he is unconscious, 1–2 mg of *glucagon* are given subcutaneously, intramuscularly, or intravenously, or 40–50% glucose solution intravenously. In general, 20–40 ml of the glucose solution are sufficient. If the condition of the patient has not improved within 2 min after glucose administration, then the intravenous injection of glucose should be repeated, and the patient rapidly transferred to a hospital, where he should be treated according to the schedule on p. 816.

As soon as the patient is able to swallow again food is given by mouth. Hypoglycemia may recur, especially with the use of long acting insulins; therefore food intake is repeated e.g. 100 cc milk every 1/2 hour for 2 hours. See p. 815 for the so-called prolonged hypoglycemic coma.

Table 25. Differential diagnosis between insulin reaction and ketoacidosis

	Hypoglycemia after		Ketoacidosis
	Rapidly-acting insulin	Long-acting insulin	
1. Causes			
Food intake	Reduced, delayed or neglected	Reduced, delayed or neglected	Dietary mistake
Physical activity	Increased	Increased	Reduced
Infections			Common
2. Previous history			
Onset	Sudden	Usually sudden	Gradually
Loss of consciousness	Rapid, within minutes	Within seconds or after minutes of increased apathy	Gradual, over hours or days
Vomiting	Seldom, only as a cause	Seldom, also as a result	Often precedes unconsciousness
Polydipsia	Absent	Absent	Severe
Appetite	Usually increased	Usually good	Poor
Visual power	Diplopia common	Diplopia common	
Rapid. pass. blindness	Diplopia common	Diplopia common	
Abdominal pains	Seldom, then only as cause	Seldom, then only as cause	Often an accompanying feature
Thoracic tension	Absent	Absent	Frequent
Diarrhea	Only as cause	Only as cause	
Mood	Restless, irritable	rarely tired	tired
3. Findings			
Breathing	Normal or snoring, irregular	Normal or snoring, irregular	Regular and deep (Kußmaul)
Smell of acetone	Absent or faint	Absent or faint	Strong
Skin	Moist, pale, normal elasticity	Moist, pale, normal elasticity	Dry, often reddened, reduced elasticity
Sweat	Increased	Increased	Absent
Tongue	Moist	Moist	Dry
Pupils	Dilated	Usually dilated	Normal or constricted
Ocular pressure	Normal, seldom reduced	Normal, seldom reduced	Usually reduced
Tremor	Present	Not always present	Absent
Musculature	Rigid	Rigid	Flaccid
Babinski	Often pos. both sides	Often pos. both sides	Negative
Blood pressure	Normal-elevated	Normal-elevated	Decreasing
Sugar (urine)	None or little	None or little	Much
Acetone in urine	Seldom present	Seldom present	Much
Blood sugar	Usually < 60 mg% or falling quickly	Low	Usually > 400 mg%
Alkali reserve	Normal	Normal	Reduced, 20 Vol% at 40 mm Hg, $pCO_2 = 9.1$ mEq/l
Leucocytes	Normal	Normal	Increased
Reaction to administration of carbohydrates	Improvement within a few minutes	Improvement usually within a few minutes	No improvement

β) Allergy to Insulin

Redness and swelling of the skin and the subcutaneous tissue at the site of injection occasionally arise after several days of treatment with insulin. These symptoms disappear spontaneously within a few weeks if the therapy is continued. There are two different types of reaction, the *immediate reaction*, occurring within minutes of the injection, and the *delayed reaction* arising only after several hours. The question of whether these are really allergic or other phenomena is still not settled. It is also still unknown whether insulin or accompanying substances are responsible for these reactions. Severe generalized *allergy* to insulin, with generalized urticaria, angioneurotic edema or with thrombocytopenic purpura has now become rare, since the modern insulins are highly purified. Patients resuming insulin therapy after interruption are particularly at risk. Because of this, when insulin therapy is resumed, the initial doses given on p. 787, should be adhered to and can be supplemented if necessary with

higher doses after as little as 30 min (see below for insulin resistance and the formation of antibodies).

The therapeutic measures consist of giving up alcohol for disinfecting the skin, and substituting pure porcine, or pure bovine, or the highly purified Nipagin insulin for the preparation used before. Mixing an antihistamine, e.g. 0.1 ml antazoline, with every injection of insulin, has proved satisfactory. After about 10 days, the insulin will be tolerated without any additive substance. For very severe cases prednisone is used.

γ) Insulin Lipodystrophy

Local atrophy of the subcutaneous tissue at or near the site of injection, or an abnormal accumulation of adipose tissue, "*insulin lipomas*" are not uncommonly found in young diabetics of both sexes, and in adult women. They are seldom seen in adult men. They occur after the use of insulin of most types and their cause is not exactly known. *Insulinfibrosis*, a condition in which the site of often repeated injections becomes hardened by the formation of new fibrous tissue, is mentioned here because it needs the same treatment. These areas are hypesthetic and the patients therefore prefer precisely these places for their injections. The lipomas and fibrosis disappear almost completely after months or years if injections are made elsewhere. On the other hand, the atrophic areas need years to be corrected. The following points have proved to be satisfactory in preventing the lipo-atrophy: replacement of the 70% alcohol for cleaning the skin by some other disinfectant such as Desogene tincture, sterilization of the syringes and needles by boiling, use of small, fine hypodermic needles (No. 0.25), and proper distribution of the site of injections (CONSTAM, 1966).

δ) Presbyopia due to Insulin

Hypermetropia develops when the eye accustomed to high concentrations of blood sugar readjusts to normal blood sugar values. It is harmless but may be alarming to the patient. The refractive disorder disappears spontaneously after 2–4 weeks.

ε) Death due to Insulin

Cases of death are very rare in comparison to the large number of patients using insulin every day. The causes to be considered are oversensitivity to insulin, and hypoglycemic shock, whether erroneous or deliberate as in suicide or murder. A mere 5 units of insulin can cause a fatal outcome in a diabetic with an unsubstituted adrenocortical insufficiency.

Resistance to Insulin. Insulin resistance is assumed when over a long period of time 200 U insulin or more are needed daily to control diabetes with a diet of roughly 200 g carbohydrates. It is, however, advisable to start the appropriate measures at the stage when a patient needs more than 120 U insulin per day and not to wait until several hundreds or even a thousand units are necessary. In certain conditions, a severe resistance to insulin can spontaneously disappear within a few months or years. However, it can also lead to ketoacidosis and death.

The following factors impair the action of insulin:

1. Delayed absorption from the subcutaneous tissue. Mild resistance to insulin may be caused by local allergy to insulin, or injections into insulin lipomas, or into fibrous or edematous skin areas. Such patients react with a prompt fall of blood sugar after intravenous administration of insulin.

2. Increased amounts of contrainsular hormones as in acromegaly, Cushing's syndrome, pheochromcytoma, hyperthyroidism, and after treatment with ACTH or glucocorticoids; thiazine diuretics can also increase the insulin requirement. The insulin resistance occasionally observed in hemochromatosis is unexplained.

3. Inhibition of the peripheral utilization of glucose, e.g. by free fatty acids, β-oxybutyric acid in ketoacidosis.

4. Blocking of insulin by antibodies, though rare, is the most common cause of true insulin resistance.

Therapy. The cells capable of forming antibodies can produce different antibodies to one and the same antigen. By electrophoresis or ultracentrifugation these antibodies may be shown to be intracellular or humoral. The predisposition to forming antibodies is genetically determined and can vary greatly from one individual to another. Intracellular antibodies can first be formed, followed by humoral ones, or only one type is produced, or both types together.

Insulin is a weak antigen in most mammals, since its molecule is so small.

Intracellular antibodies to insulin lead to delayed allergy, whilst humoral ones produce immediate allergic reactions, rarely anaphylaxis, sometimes neutralization of the insulin of all mammals including the human, except for the

guinea pig. The insulin-antibody complex can partly be precipitated in the cold, and then at a strongly acid pH insulin may be reliberated from it. For reasons unknown, the antibody titer can rise at one time, and fall at another, and in addition its binding capacity for insulin can vary.

3 weeks to 3 months after treatment is started with bovine insulin, antibodies to insulin, which can bind in general about 10 U insulin/liter plasma are found in diabetics as well as in non-diabetics. This occurs less frequently with porcine insuline. In cases of insulin resistance, these antibodies can be greatly increased and can fix several thousands of units/liter plasma. The severity of the resistance, i.e. the insulin requirement, for unknown reasons does not run parallel to the levels of antibodies contained in the plasma. In the presence of high antibody titers, soluble insulin can be regained and steadily liberated again, so that it acts like a depot insulin over 24 hours or more (FANKHAUSER). However, in patients resistant to insulin large amounts of insulin can also be suddenly liberated again, for unknown reasons, and can lead to severe hypoglycemia.

If the diet is adequate, and both infections and an endocrine hyperfunctional syndrome, hyperthyoidism in particular, can be excluded, then porcine soluble insulin must first be used in cases of insulin resistance, instead of the bovine or mixed insulins, since it is less antigenic in the human. If this has no effect when given subcutaneously, 20 U are given i.v., which normally causes the blood sugar to fall by 50%, and which will confirm or exclude a disturbed subcutaneous absorption.

Oral antidiabetic agents, the sulfonylurea derivatives or biguanides, can be tried in combination with insulin, as a third measure. The molecular structure of altered insulins has a less antigenic effect, and recently a sulfated insulin (ARNOTT, 1965) has been recommended for use in insulin resistance. Only after all these measures have proved futile, a trial with prednisone is indicated, but this should only be done under the supervision of an experienced specialist. At first 60–80 mg of prednisone are given daily. Success usually becomes apparent only after a few weeks. In certain conditions a small maintenance dose of 5–10 mg must be continued for a very long time. When these measures fail, the insulin therapy must be continued boldly with massive doses of insulin; using preparations with concentrations of 100, and even 500 U per ml. Careful supervision is essential, since the insulin resistance may suddenly disappear any day.

7. Accompanying Symptoms and Complications of Diabetes Mellitus

Diabetes mellitus is accompanied by characteristic tissue changes, the majority of which do not become clinically apparent until 8–20 years after the first clinical manifestation of the disease. LUNDBAEK used the phrase "long-term diabetes syndrome" to summarize these changes. This syndrome is largely due to microangiopathy, which, on careful examination, can already be found in the early stages of diabetes. In a few cases, the corresponding clinical symptoms may even precede clinical diabetes, so we can appropriately replace the term "long-term diabetes syndrome" by "diabetic angiopathy" and "diabetic neuropathy".

The histologists differ in opinion about the question of whether this is a change specific to diabetes, or arteriosclerosis, modified by diabetes. The alterations in the retina, and the nodular form of glomerular sclerosis are recognized by the majority of pathologists as being characteristic of diabetes. Although the arterial changes show no histological differences, their location is typical in diabetes. In addition to retinopathy and nephropathy, peripheral angiopathy in the lower extremities seems to be typical. Histochemical investigations on the aorta, on coronary and peripheral vessels, have shown differences between arteriosclerosis and diabetes, but the significance of this is uncertain. According to GOLDENBERG and BLUMENTHAL, changes characteristic of diabetes are mainly found in the arterioles and venules with a diameter of less than 150 μm. Hypertrophy and hyperplasia of the endothelial cells, among which fine PAS-positive fibrils are embedded, are typical, as is thickening of the basal membrane.

Involvement of the smaller vessels with exemption of the larger arteries are clinically and pathologically considered indicative of diabetes, and not of arteriosclerosis. This suggests specific vascular changes in diabetes, but mixed forms are common.

Is diabetic angiopathy a result of diabetes mellitus, or is diabetes the consequence of vascular disease? The facts supporting the first view are: the vascular disorders increase in number and severity with the duration of diabetes, the long-term diabetes syndrome has been observed after pancreatitis, after pancreatectomy, in hemochromatosis, and development of the long-term syndrome can be delayed by early control of diabetes. The facts speaking against this view (which are, however, being questioned again) are: observations of retinopathy or peripheral angiopathy or necrobiosis

lipoidica, of microscopic vascular changes, before the diabetes becomes overt, and even in the conjunctivae of healthy children of diabetic mothers. Finally a still unknown factor X has been postulated, which produces both the vascular changes, and diabetes. However, in recent years it seems more and more probable that the angiopathy is the result of diabetes.

The micro-angiopathy occurs in all organs, but is clinically preponderant in the eyes, the kidneys, the peripheral, cerebral, and coronary vessels, and also in the nervous system.

In contrast to angiopathy, infections, allergies, tumors, and injuries may be incidental accompanying features not related pathogenitically to diabetes mellitus, although their courses are considerably influenced by diabetes.

a) Changes in the Eyes

Ptosis of the upper eyelid is not uncommon in diabetics, even without hypothyroidism. *Microaneurysms of the conjunctival capillaries* are significantly more frequent in diabetics and in the non-diabetic children of diabetic mothers, than in the healthy descendants of non-diabetics. A stripe-like clouding of the *cornea* occurs in particular with dehydration in diabetic coma. *This folding of the membrana Descemeti* is the result of reduced intraocular pressure. The loosening of the pupillary margin is characteristic of diabetes. *Diabetic iritis*, a toxic form of iridocyclitis, differs from the inflammation of the iris in the absence of precipitates. The rare *rubeosis iridis*, a red mottling of the iris produced by the expansion and new formation of capillaries is usually followed by malignant hemorrhagic glaucoma.

See p. 793, 794 for disorders of the pupils and the ophthalmic nerves.

Refractive changes are common. Every case of myopia starting after the age of 30 is suggestive of diabetes, whereas hypermetropia arising suddenly, during treatment of diabetes, indicates hypoglycemia. Insulin-induced presbyopia has already been mentioned on p. 790.

In diabetic coma, turbidity of the lens may arise as a result of dehydration, and disappears 1–2 days after the ketoacidosis has been corrected. *Thickening of the anterior fission area of the lens* may be found in young patients 2–3 years after the diabetes becomes overt. This rarely occurs in non-diabetics. The senile cataract possibly arises somewhat earlier in elderly diabetics than in non-diabetics. Treatment is the same. In contrast to this, the *diabetic cataract*, begins subcapsularly, and may occasionally develop within a few hours to days, especially in patients under 50 years. It is relatively infrequent and is encountered only in neglected cases of juvenile diabetes. Antidiabetic therapy may, but does not always, succeed in making this turbidity disappear. In some cases, it may, therefore, be necessary to remove the diseased lens surgically. Primary and secondary glaucoma appear to occur more frequently in diabetics, even in young patients, than in non-diabetics.

Diabetic retinopathy is often the long-term symptom which is first recognized, because it can be diagnosed by simple means. Regular examinations are an important part of long-term care of diabetic patients. The earliest changes and the evolution of the condition can best be demonstrated by repeated fluorescine angiography. Fortunately, central vision in the majority of cases is maintained for many years. The formation of new blood vessels, *proliferative retinopathy*, is an ominous sign, often followed by *hemorrhage into the vitreous body* leading to *detachment of the retina*. Even in the absence of visible retinopathy, diabetics appear to be more predisposed to detachment of the retina.

The development of the retinal changes depends on the duration of the illness but also, to a considerable degree, on the time of onset and quality of the treatment. The intermittent course of retinopathy – remissions are not uncommon during the early stages – makes it difficult to assess the effect of therapeutic measures in short-term studies. Careful treatment can lead to the disappearance of hemorrhage and exsudates in patients under 60 years of age. The *prognosis* for elderly patients and in cases with proliferative retinopathy is doubtful. The large series of statistics presented by White shows that of the 1000 or more diabetics, 6% were blind when the metabolic disorder had been present for 20 years or longer. Therefore, prevention is very important, and the judicious treatment of diabetes should be instituted as early as possible.

Hyper- and severe hypoglycemia are to be avoided since the latter is said to provoke hemorrhages into the vitreous body. The diet should be low in fat, particularly in saturated fatty acids. Exertions which increase congestion in the head, particularly straining while holding one's breath, are to be avoided. The large number of recommended vitamin flavonoid and lipocaic preparations have so far not proved very effective.

The action of drugs causing a fall in the blood lipids has not been confirmed, nor has treatment with sex hormones and anabolic androgens stood up to precise testing. Anticoagulants, corticoids and ACTH are contra-

indicated. The observation that the retinopathy disappeared with the development of Sheehan's syndrome led to the recommendation of hypophysectomy (see p. 115) or the division of the pituitary stalk in selected patients. The mode of action is unknown. Loss of growth hormone is, according to some authors, not responsible for the effect (POWEL, 1966). On the other hand, pooled findings indicate that 1/3 of the patients benefited from the procedure, but the indications were strict in these cases. 2/3 are improved, but spontaneous improvement or stillstand can also occur in 1/3 (RUCKER, 1967).

The situation must be carefully considered, before this procedure is recommended, as it necessitates life-long substitution with cortisone and eltroxine. A certain degree of intelligence and responsibility in the patient are required. The diabetes needs less insulin afterwards, but becomes more labile and more prone to severe hypoglycemia. The transnasal stereotactic electrocoagulation of the pituitary (see p. 116) does not cause much stress and the patient can get up on the 2nd day. Hypophysectomy is only indicated in patients with progressive retinopathy despite excellent diabetes control; the eye sight of the better eye must still permit sufficient vision for an active life, the patient must be intelligent enough to handle his diabetes despite its tendency to marked hypoglycemia, and the substitution for the loss of adrenal, pituitary and sometimes sexual functions. *Nephropathy* is a contraindication to this operation. Last but not least, the patient must be willing to take the risk of this complicated treatment, which does not improve life expectancy. A reliable presentation of the indications and contraindications will only be possible after more experience of this method of treatment.

Retinal lipemia is rare and found only in forms of lipemia and in severe ketoacidosis. It does not influence the visual power, and disappears after intensive treatment of the diabetes with insulin and a fat-restricted diet. All ophthalmologists recognize the specificity of the *diabetic pigmentopathy of the macula lutea. Retrobulbar neuritis* is not specific to diabetes. *Atrophy of the optic nerve* has occasionally been observed even in young diabetics.

b) Diseases of the Nervous System

α) Emotional Disturbances

Depression can be caused by the patient's realization that he has an incurable disease, by increasing fatigue and weakness and by the possible association of sexual impotence. On account of this, patients with severe diabetes often assume a hopeless attitude at the onset of treatment. However, after a few days of intensive therapy when strength and hope of surviving are restored, this mood changes.

Diabetes mellitus can be found combined with all types of functional and organic disorders of the nervous system. According to BLEULER, there is a positive correlation with manic-depressive illness, arteriosclerosis, and senile psychoses, and a negative correlation with schizophrenia, oligophrenia, and possibly also with epilepsy. In any case, depression occurs frequently, and we have the impression that the duration of the depressive phase can be shortened by good control of the diabetes.

β) Disturbances of the Sense Organs

The visual disturbances have been discussed in Sect. a. *Olfactory disturbances* are demonstrable in over 60% of long-lasting cases of diabetes. The *taste* threshold is elevated for glucose but normal for table salt. *Impaired hearing* without inflammatory changes in the ears have been confirmed even in young diabetics. The acoustic nerve is selectively involved, the vestibular nerve almost always spared.

γ) Diabetic Neuropathy

This term includes peripheral, central, radicular and medullary symptoms, as well as those of the autonomic nervous system. A really satisfactory classification is not possible, since this condition has an exceptionally polymorphous symptomatology, its pathogenesis is not clear and the histology is inconsistent. Depending on the predominant clinical features, the terms mono- or polyneuropathy, encephalopathy, enteropathy, radiculopathy and so on are used. The old description "diabetic pseudo-tabes" remains a fitting term for some cases.

Pains arise mainly in the lower extremities, often on both sides, sometimes symmetrically. They are described as dull, cramp-like, tearing or burning; they typically occur during the night and disappear when the patient gets up and walks. They are preceded long in advance by a phase of paresthesia, a feeling of coldness, numbness, prickling or burning. Hyperesthesia may become tormenting, and anesthesia can lead to severe burns.

The objective findings are diminution or loss of tendon reflexes, especially of the ankle and knee jerks, and reduction of the sensation for vibration, especially in the feet. There are also hypesthetic and anesthetic zones, mainly in the lower limbs, and in certain conditions

there is an increased sensitivity to pressure of all the muscles at their points of insertion. The ankle-jerk reflexogramm is retarded in the absence of hypothyroidism, and the conduction rate of the motor nerves is delayed even in young diabetics.

Weakness of the peroneal muscles is not infrequent, but paresis or paralysis of any other muscle or of any of the cranial nerves with the exception of I and XII do occur. The cranial nerves III and VI are involved most frequently. In contrast to pains and paresthesia, paresis and paralysis are usually unilateral.

The *amyotrophic form* of diabetic neuropathy causes atrophy of the pelvic and pectoral girdles in particular, but also of the muscles of hand and feet.

In exceptional cases, all these symptoms can precede the clinical manifestation of diabetes.

Pupillary disturbances are common, but a typical Argyll-Robertson reaction is rare. The atony of the stomach – *diabetic gastroparesis* – leads to delayed emptying of the stomach and to retention of food, making good control of the diabetes impossible. This is occasionally accompanied by *diabetic enteropathy*, with its nocturnal or postprandial diarrhea (*diabetic diarrhea*) or nocturnal incontinence of stools or severe chronic *constipation*. Objective findings are peripheral neuropathy, sometimes accompanied by symptoms involving the autonomic nervous system, such as *abnormal sweat secretion*, orthostatic or positional *hypotension* and *diabetic arthropathy*. Intestinal X-rays may show a so-called malabsorption picture and/or a large hypotonic stomach (gastroparesis diabeticorum). The differential diagnosis has to exclude atony of the anal sphincter, exocrine pancreatic insufficiency, and sprue.

Dystonia of the urinary bladder due to weakness of the detrusor muscle can be recognized cystometrically and may lead to atony of the organ. Early recognition of this condition is important in order to prevent secondary infections.

The neurogenic forms of impotence, sometimes associated with retrograde ejaculation, is another manifestation of diabetic neuropathy.

Callosities of the feet, usually localized over the heads of metatarsal bones, are generally painless. They develop even in feet with well preserved circulation. They often precede *trophic ulcera*, the so-called "mal perforans".

Neuropathic joint changes analogous to CHARCOT's arthropathy are found in the ankle, the tarsal and the tarso-metatarsal joints, rarely in the knee. They are clinically characterized by sometimes extensive destruction of bones and joints in the absence of osteoporosis, osteomyelitis or infection. The blood supply is sufficient. Swelling occurs in the absence of inflammation, with neither tenderness nor temperature changes prominent.

Diabetic osteopathy, mainly localized in the feet, reveals spontaneous fractures and/or atrophy of the phalanges on X-ray examination.

Minor injuries, repeated and unnoticed for a long time by the hypalgesic patient, are considered the *cause* of callosities, arthropathy and osteopathy (p. 799, 800).

Disturbances of the autonomic innervation can cause inactivation of the sweat glands, loss of vaso- and pilo-motor functions, reversal of temperature gradients of the skin, formation of *edema*, *orthostatic hypotension* with or without *orthostatic tachycardia*, and *night sweats*, in circumscribed areas of the body only.

Most patients with diabetic neuropathy present an increase in the protein content of the *cerebrospinal fluid* with normal cell count.

There are no pathognomonic signs for diabetic neuropathy, so that diagnosis must be reached by a process of elimination. Therefore, the differential diagnosis deserves close consideration.

The relations between the diabetic neuropathy and the severity of the metabolic disorder are not clear; with longer duration neurological changes increase in frequency.

The prognosis is good for the majority of patients with paralysis. Paralysis involving the cranial nerves generally recedes in 6–12 weeks. The amyotrophic forms of the pelvic and shoulder regions regress within 3–18 months, in contrast to those involving the distal parts of the lumbs, hands and feet, where there is little chance of recovery. The prognosis is unpredictable for neurogenic impotence and diabetic enteropathy. Urinary bladder atony in young diabetics may disappear when good control of the diabetes is achieved. In elderly people, cholinergic drugs, e.g. carbachol up to 16 mg daily, can be tried. If this fails, resection of the internal sphincter of the bladder seems indicated. Opium in the form of drops, or Reasec, and disinfection of the bowels with sulfonamides, neomycin or chloramphenicol have been recommended for diabetic diarrhea.

The effects of sodium dithiocaprylicum and vitamin B_{12} on the pains are still not clear. The former drug is injected intravenously in a dose of 40–50 mg twice daily for one week, and is repeated if indicated. 1000 γ of vitamin B_{12} are given intramuscularly for five days. Good control of the diabetes is perhaps the most important factor. Insulin also appears to be more effective here than the oral antidiabetic agents.

In addition to treatment with diet, insulin and vitamins, orthopedic measures may be necessary in some cases of arthropathy to obtain reasonable equilibrium.

c) Disorders of the Cardiovascular System

The larger arteries of diabetics present a more extensive and severe atherosclerosis than the vessels of non-diabetics of the same age group. Heavy calcifications are found more frequently in the medium-sized arteries, and the obliterative endothelial proliferation is most striking in the arterioles. Swelling of the endothelial cells and thickening of the basal membranes are typical findings in the capillaries. This microangiopathy seems to affect all tissues of the body.

The relations between diabetic angiopathy and arteriosclerosis and the development of vascular changes prior to clinical diabetes have been discussed on p. 791. The connections between fats, proteins, lipoproteins and glycoproteins of the blood, with diabetic angiopathy have still not been fully explained.

In young diabetics, the angiopathy generally first becomes clinically apparent in the eyes and kidneys; in elderly patients disorders of the heart, lower extremities, and brain are predominant.

α) Peripheral Vascular Disease

Every peripheral circulatory disorder demands a careful examination for diabetes. The clinical features, with the exception of a few peculiarities (frequency of gangrene and preference for the smaller peripheral vessels), do not differ from those of arteriosclerotic or endarteritic angiopathy. They can be found in monographs (RATSCHOW, 1959; KAPPERT, 1960). They are usually treated medically and surgically in the same way, with the exception of the optimal control of the diabetes.

The true form of diabetic angiopathy is found particularly in the lower extremities. It can also arise combined with or released by infections in the upper limbs, in the skin, in the face and in the vulva or penis when the diabetes is uncontrolled.

Tissues of an extremity with a poor blood supply are easily injured. This is true particularly in diabetics with diminished sensitivity for pain and heat as a consequence of their neuropathy. Slight injuries, foot mycosis, corn plasters, too hot a foot bath or exposure to cold can produce ulceration on the feet. As soon as the continuity of the skin is interrupted there is danger of infection.

Fissures, sometimes very painful, are mostly localized on the heels and are often produced by putting on shoes without unlacing them sufficiently.

Gangrene, the necrosis of tissue, follows the obliteration of a vessel, and may be precipitated by mechanical, chemical or thermal trauma, or by infection.

Prevention is better than cure. Prevention of diabetic angiopathy consists in establishing precise control of the diabetes as soon as possible, in maintaining this control and avoiding injuries. Protection from excessive heat and cold, good care of the feet, treatment of foot diseases and deformities are important prophylactic factors.

Since the introduction of insulin, gangrene arises on average 10 years later than previously. The importance of good treatment of the diabetes can not be overemphasized. Insulin appears to be more effective than oral antidiabetic drugs.

We use vasodilating drugs orally, intramuscularly or intravenously, to improve collateral circulation. Sometimes, these drugs are given as an intra-arterial injection or infusion. The value of the long-term treatment with anticoagulants has not yet been proven. Total abstinence from nicotine is important. When indicated, the patients are referred to a vascular surgeon for a unilateral lumbar sympathetic ganglionectomy which may be combined with excision of short sections of obliterated arteries or with endarterectomies. Diabetic neuropathy finally leads to suppression of the peripheral sympathetic function. In spite of this, we use lumbar sympathectomy in earlier stages, which then increases peripheral circulation considerably. The whole treatment of peripheral angiopathy is a fight for time; if we can postpone amputation for some years, a surgical intervention is justified. If the block lies in the femoral artery or higher, bypass operations have proved useful even in diabetic patients. The use of baths, massage, leeches and in particular local treatment with heat or electric currents must be avoided, since they can precipitate gangrene. In general, mummified tissue areas are not excised, and it is advisable to wait for spontaneous segregation. Chemotherapeutic agents and antibiotics are used in the usual dosage. In some conditions, they are applied intra-arterially and/or locally. The local application of trypsin helps to remove necrotic or indurated tissue. In general, we try to keep the affected area as dry as possible.

In infected gangrene major surgery has to be postponed until the diabetes is controlled and general resistance improved. Till then, surgical intervention is limited to draining an abscess or removing a superficial crust. With the help

of quick-acting insulin, antibiotics, and perhaps blood transfusions the patient's condition improves in a few days, whereupon surgical elimination of the entire infection becomes feasible.

However, in the presence of a fulminating, extensive infection, surgery cannot wait so long. A few hours of active insulin therapy, if necessary intravenously, by an experienced physician, and hypothermia to the affected area considerably improve the patient's chances in the emergency surgical intervention.

An amputation is only justified if gangrene is progressing despite all therapeutic measures, optimum control of diabetes with insulin, if the pain does not become tolerable within a few days, or if there is any danger of septicemia.

Any decision on amputation must be made in the knowledge that a patient with severe diabetic angiopathy usually has myocardial damage, which will make the use of an artificial leg difficult and perhaps impossible. Even after a small, partial amputation the other extremity will have to carry more weight. Will it be able to do this despite the angiopathy which is always bilateral? In our opinion, the *most restricted amputations* are only possible after the diabetes is well controlled, but even then we must be prepared to take the risk that further amputations higher up may later become necessary.

McKittrick's advice has stood the test, and atropine must be the only premedication, no local anesthesia, no tourniquet; the stump should be left entirely or partly open after amputation for infection, and a drain should be inserted only when the infection has not been removed. The stump should be closed without a drain after amputations for non infected arterial insufficiency.

Good control of diabetes by diet and insulin with sugar determinations at different times of the day is necessary and will permit good healing of the amputation stump. In addition, any anemia or hypoproteinemia must be corrected.

Thrombophlebitis in the diabetic is treated in the same way as in subjects with normal metabolism, providing the diabetes is well controlled.

β) Hypertension

Hypertension is more common in diabetics than in non-diabetics. Even in growth-onset diabetics, blood pressure is frequently found to be elevated after 20–25 years' duration. Hypertension appears earlier and more frequently when the diabetic treatment is inadequate. Elevation of blood pressure in a young diabetic should always suggest diabetic nephropathy, but one should also consider acute glomerulonephritis, Conn's syndrome and pheochromocytoma. Myocardial infarctions and gangrene are more common in hypertensive than in normotensive diabeties.

The use of salidiuretics of the benzothiadiazine group or of chlorthalidone to reduce blood pressure, may necessitate an increase in the dosage of insulin in some conditions. See p. 794 for orthostatic hypotension.

γ) Heart Disease

Diabetes is very often accompanied by aortic and/or coronary sclerosis. This occurs in diabetic men and women with equal frequency whilst it is rare in non-diabetic female patients.

The frequency of coronary sclerosis and thrombosis increases with the duration of diabetes and with poor quality of treatment. Myocardial infarction in a woman before the menopause suggests diabetes mellitus. *Coronary thrombosis* is the most common cause of death in elderly diabetics.

The *prognosis* of coronary sclerosis is greatly dependent on the quality of the antidiabetic treatment.

Reduction of weight in overweight patients, the exclusion of nicotine, and removal of infections, especially of the urinary tract, are important. Reduction of body weight and of blood sugar must be carried out cautiously. The diet should be poor in fats but relatively rich in carbohydrates. Hypoglycemia must be avoided, since severe falls in the blood sugar can precipitate myocardial infarction. Patients with heart disease often feel better with a slightly elevated than with normal blood sugar levels. Therefore, the insulin dosage must be carefully, adjusted considering also the subjective feeling of the patient.

d) Diseases of the Kidneys and the Urinary Tract

The collective term "*diabetic nephropathy*" appears to be appropriate since the renal changes found in diabetes mellitus are so polymorphous. It is now the most frequent cause of death in growth-onset diabetics.

Intercapillary glomerulosclerosis was described by Kimmelstiel and Wilson as characteristic patho-anatomical changes in the kidneys (see p. 768). Both glomerular and tubular functions are limited. Glomerulosclerosis is found in diabetics who have been treated with insulin and in others who have never received this hormone. The duration of diabetes, and possibly its intensity, the quality of treatment, the stage

at which it was started, and urinary-tract infections influence the development and course of glomerulosclerosis.

The *diagnosis* of glomerulosclerosis is a histological one. During life it can only be confirmed by renal biopsy. The condition can be suspected when there are clinical symptoms of a nephrotic syndrome, such as albuminuria without particular findings in the sediment (apart from the bi-refractive lipoids [Maltese crosses]), hypoproteinemia with edema and hyperlipemia, associated with hypertension, azotemia, and retinopathy; sometimes only a few of these symptoms are present.

The prognosis is bad. We know of no means which will halt this condition, which lasts for 2–12 years, on average 6–7 years.

Treatment is directed at trying to eliminate any accompanying infection. As long as there is no retention of nitrogen, one aims at compensating the protein loss as far as possible with a diet relatively rich in proteins and poor in sodium. In the presence of azotemia, the protein intake must be reduced, but not below 0.5 g/kg body weight. In uremia, numerous different methods of estimating the blood sugar give values which are too high.

The terminal phase of the condition is occasionally accompanied by a striking improvement of carbohydrate tolerance. Various factors have been suggested as the cause of this: delayed elimination of insulin through the urine, accumulation of the guanidine bodies, increased permeability of the cells, facilitating the transport of glucose into the tissues in the absence of insulin, increased excretion of proteins binding insulin, and reduction in the caloric intake.

Diabetics seem more susceptible to infections, which frequently involve kidneys and urinary tract. There may be inflammation of the renal pelvis, the ureters, the bladder, the ovaries, and the urethra, as well as the formation of abscesses in the perirenal tissues, in the kidneys, and the prostate etc. Some of the former infections may easily be missed if they cause no subjective symptoms.

The *necrosis of the renal papilla* is fairly typical of diabetes mellitus. It affects women more frequently. The diagnosis must be considered if severe renal symptoms suddenly arise or when a not very active pyelonephritis leads to high pyrexia, hematuria and renal colic, with no other apparent reasons. There may sometimes be particles of renal tissue in the urinary sediment. A retrograde pyelogram shows changes which are more or less characteristic. However, the patient's grave general condition often prevents radiological examination. The only therapeutic measure possible is to combat the accompanying infection with antibiotics.

When the urine is infected, the results of the tests for sugar in the urine are unreliable. *Pneumaturia* is occasionally seen in diabetes. The cloudy urine, looking like apple juice just off the press, contains gas. The patients complain of irritation of the bladder, and of inability to void urine while lying. If the pneumaturia does not disappear after the blood sugar is normalized, atony of the bladder and vesicovaginal or rectovesical fistulae have to be considered.

Dystonia and atony of the bladder: the interval between emptying the bladder increases until the patient must only void urine twice in 24 hours, and then under pressure. Examination reveals residual urine in the absence of obstruction and cystometric hypotension (see Sect. γ).

e) Infections

Diabetics are especially suspectible to infections, which usually increase the insulin requirement. On the other hand the tolerance may improve in extreme conditions with collapse and inanition. Poorly controlled patients are less resistent. With the exception of draining superficial abscesses without anesthesia, surgical procedures must be postponed until the diabetes is under control.

Furuncles and *carbuncles* are so common in untreated diabetics, that their occurrence in non-diabetics must lead to a test for diabetes.

Before insulin was introduced in the treatment of diabetes, many diabetics died of *pulmonary tuberculosis*. The incidence of pulmonary tuberculosis is still twice that in non-diabetics. The flaring up of a tuberculous infection is usually but not always followed by a deterioration of carbohydrate tolerance to the extent that ketoacidosis may develop. Sometimes, the latter precedes the former.

The prognosis of tuberculosis in diabetes has become considerably more favorable since the introduction of insulin, and in particular since chemotherapeutic agents and antibiotics have become available.

Mycosis of the foot is without doubt the most common infection which can precipitate or worsen gangrene. Candida albicans (thrush) is sometimes the cause of *vaginitis* or *balanitis* in diabetics. *Pruritus vulvae* in diabetes is suggestive of fungal infection.

f) Diseases of the Skin

See above about infections. They are treated in the same way as in non-diabetics. A rosy skin on the forehead and cheeks, and sometimes

also on hands and feet is von Noorden's *rubeosis*, an early sign of poorly controlled diabetes. It is caused by dilatation of skin capillaries.

Xanthomatosis, the diabetic xanthoma, may occur, as in any other form of hyperlipemia, in poorly controlled diabetics. They can appear as a dissemination of yellowish, sometimes also reddish, occasionally pruritic or even painful, papules and plaques, which are distributed symmetrically over neck, back, buttocks, elbows, and knees. A low-fat diet and insulin cause the diabetic xanthoma to disappear.

Xanthelasma, i.e. the deposition of lipoids in the eyelids has no connection with diabetic xanthoma. It may be more frequent in diabetics than in non-diabetics.

Tornblom was the first to discover, and Melin the first to describe *diabetic dermopathy* or *circumscribed diabetic skin atrophy*, or the "*spotted leg syndrome*". All these terms depict changes in the skin below the knees on the anterior and lateral aspects of the lower legs and very rarely on the extensor surfaces of other limbs. At first, multiple, reddish, flat papules arise, round to oval in shape, measuring 5–12 mm across and sometimes arranged in a line. Some papules show a central depression and blisters may form. Later, this skin eruption becomes crusted and the typical picture of the disorder does not develop until after a few months; the skin where the papules had been becomes brownish, slightly depressed and covered by an atrophic epidermis. These atrophic areas disappear spontaneously after 1.5 to 2 years.

The differential diagnosis includes freckles and pigmentation due to varicosis.

The cause of these skin changes is not definitely explained. No treatment is necessary.

Diabetic necrobiosis lipoidica occurs mainly in the younger diabetics and particularly in women. In this condition, there are round to oval-shaped plaques, sharply demarcated, with rather shiny atrophic centers. The peripheral areas have a yellowish or reddish colour. As a rule, the skin eruptions occur symmetrically on both sides, and are rarely unilateral. The plaques are sometimes preceded by a stage with reddish papules of only a few mm in diameter, which may increase to 2 cm and often undergo necrosis in the middle. The small ulcers heal after a few weeks and leave dark pigmented scars which may reopen from time to time. The cause of this disorder is unknown. The ulcers heal when the diabetes is well controlled, but not the plaques, which may increase in size. Isoniazid is used successfully in the majority of patients (DEGOS, 1966). If it fails, we use local cortisone creams and injections of diluted solutions of corticoids under the affected skin.

Gray or dark brown pigmentations of the skin suggest hemochromatosis. See Chap. XVIII for diabetes mellitus combined with Addison's disease.

Insulin-induced lipodystrophy has been discussed in Sect. d, and the skin changes during treatment with the sulfonylurea derivatives in Sect. c.

A thickening of the subcutis in the palm of the hands is rarely met in diabetes of long duration. There is a loss of the elastic fibers, but no signs of collagenosis. This *stiff-hand syndrome* (LUNDBAEK) inconveniences the patient especially in the early morning.

g) Diseases of the Digestive System, Including the Liver

Acute pancreatitis can give rise to transitory hyperglycemia and glycosuria with diabetes-like glucose tolerance tests. According to MILLER, 2% of the cases are followed by true diabetes mellitus. *Chronic pancreatitis*, on the other hand, is considered to cause diabetes mellitus more frequently – 13% of the cases of chronic pancreatitis without the formation of calculi, and 45% of the cases associated with calculi formation result in diabetes mellitus. If the period of observation is extended to more than 18 years, about 10% of cases of *necrosis of the pancreas* may develop diabetes (BERNHARDT). Conversely, acute pancreatitis can lead pre-existing diabetes to develop rapidly into keto-acidosis.

Carcinoma of the pancreas is 3–4 times more frequent in diabetics than in non-diabetics.

Hepatomegaly is a sign of poor control, especially in young diabetics. The histological findings reveal steatosis in the majority of the cases, and glycogen infiltration in a few cases. Good control of the diabetes results in the rapid regression of the enlarged liver. Overdosage of insulin for many years is said to produce glycogen infiltration.

h) Diseases of the Sexual Organs

Menarche arises about a year later in diabetics, and the *menopause* occurs a few years earlier than in non-diabetics. Hormonal *contraceptives* worsen the metabolic state only slightly, however, and perhaps only temporarily. Today, the *fertility* of well controlled diabetic young men and women is almost normal. However, there is a precocious loss of potency in the male, even during middle age, sometimes followed by loss of libido. A reduction of libido and of the

capacity to reach orgasm is met in diabetic women too, although much less frequently than in men.

Temporary impotence due to the loss of strength in uncontrolled diabetes disappears with proper antidiabetic treatment. We more frequently encounter *chronic impotence* on account of weakness or lack of erection. This usually occurs in diabetics with peripheral angiopathy. More rarely, so-called "retrograde ejaculation" is found as a consequence of diabetic neuropathy, i.e. an increasing amount of the ejaculate, and finally all of it, flows back into the bladder, due to insufficiency of the internal sphincter. Large numbers of sperms can be found in the urine voided thereafter. In addition to these vascular and neurogenic factors, psychic stress and hormonal disorders may also be of importance. SCHÖFFLING and his team found that the testes were reduced in size in almost 1/4 of the impotent diabetics, and that the prostate was enlarged in about a third. Hypogonadotropic hypogonadism, however, has not been confirmed with certainty. The prognosis of neurogenic impotence is poor, it is doubtful in angiogenic impotence in patients over 50.

The Klinefelter syndrome, though rare, is sometimes found combined with diabetes mellitus. The same applies to gonadal dysgenesis and to Turner's syndrome (see p. 713). The cause of these combinations is unknown.

Vulvitis or balanitis are often the first symptoms leading to the detection of diabetes.

i) Diseases of the Other Endocrine Glands

Refer to Chap. XVIII for diabetes in other endocrinopathies.

k) Diseases of the Blood and of Hemopoiesis

The pale face of many patients with diabetes of many years' standing is not due to anemia, but rather to narrowing of the cutaneous vessels. *Anemia* can, however, easily be overlooked due to rubeosis or xanthosis. There are no morphological changes of the blood picture typical to diabetes mellitus. *Pernicious anemia* is not uncommonly found combined with diabetes. This combination occurs too frequently to be explained by mere coincidence, and it is striking that both diseases can be familial and can also arise in combination with hypothyroidism and with the nontuberculous Addison's disease. This may indicate that the disease is an autoimmune illness (see Chap. XVIII). Diabetics more often suffer from a *normochromic macrocytic form of anemia* which is difficult to treat.

l) Diseases of the Bones and Joints

The term "*diabetic foot*" describes a swelling in the region of the ankle joint, which occurs infrequently, develops slowly without pain, and results in a typical valgus position of the externally rotated foot. Another form which develops acutely with pain and reddening of the skin and with a rise in temperature is considerably less frequent. The diabetic foot is due to a diabetic arthropathy and osteopathy of tarsal bones.

More frequently, the *diabetic osteopathy* is found in the metatarsals and the phalanges where the diaphysis joins the epiphysis. The distal regions of the metatarsal and the proximal areas of the phalanges are the most common sites for these changes. Often osteopathy is accom-

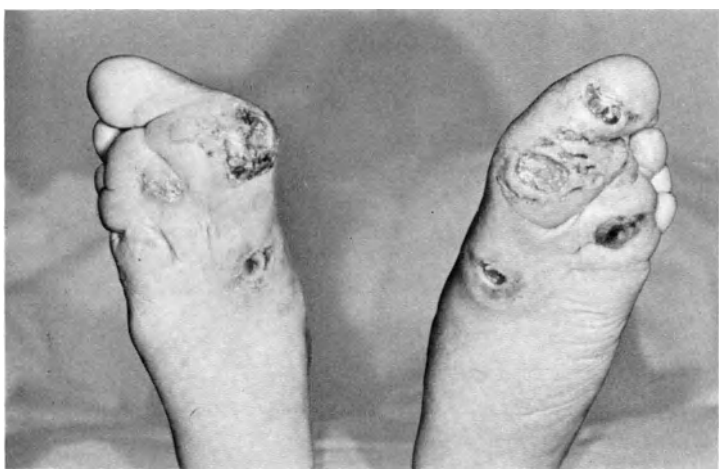


Fig. 18a. Diabetic foot

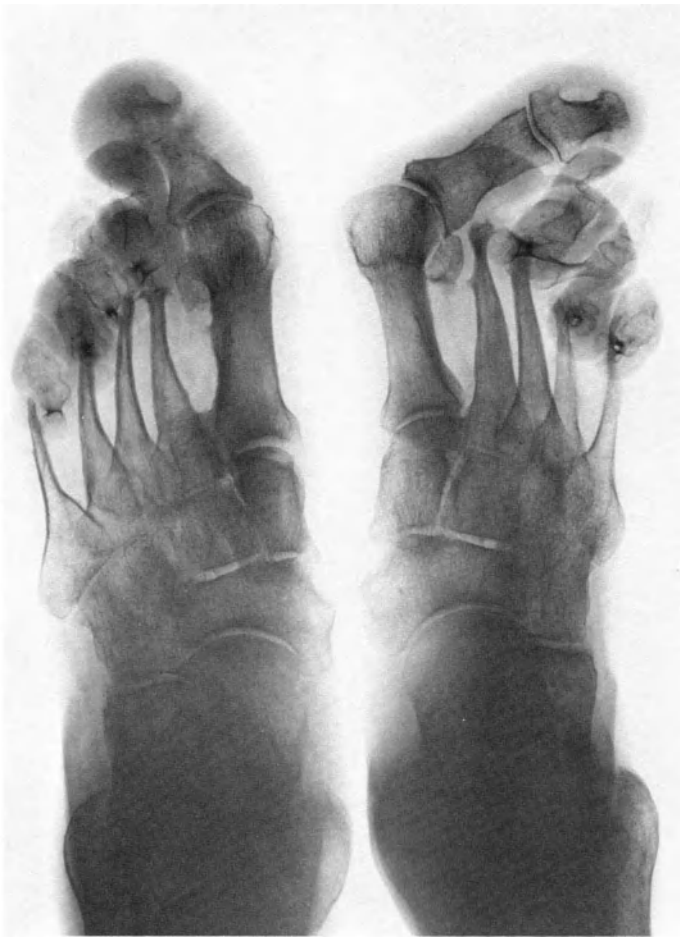


Fig. 18b. X-ray of the diabetic foot

panied by trophic ulcers, – mal perforant – or by its preceding stages, and by thickening of the skin areas exposed to great pressure. These concomitant features are not the cause but the results of the bony changes. The bones are osteoporotic and there are signs of osteolysis which leads to fragmentation or rupture of the cortex and to secondary changes in the joints, sometimes with incomplete dislocation. This displaces the toes, dorsally and backwards, leading to noticeable shortening of the foot. Reparative periosteal reactions with the formation of new bone by the periosteum or osteophytes are rare.

Clinically there is relatively little pain. Often these symptoms are combined with diabetic angio-, neuro- and retinopathies. Recurrences in the same or in the other foot, sometimes after years, are common.

The cause of the diabetic osteopathy is not quite clear and perhaps not always the same. Neurogenic disorders cannot explain all the symptoms. In the American medical literature, the term Charcot's joint is used, suggesting a neurogenic mechanism. In addition, angiopathic changes, and in some cases infections or repeated injuries of hypalgetic tissues may be involved. The fact that the condition is usually localized in the lower extremities stresses the importance of mechanical strain.

The diabetic foot is treated by orthopedic measures. Parsons has recommended lumbar sympathectomy. The bone fragments loosened by osteolysis are sometimes spontaneously cast off, leaving a healed but shortened toe.

Osteoporosis is frequently seen in adult diabetics, and at an earlier age than in healthy people. We suspect that a diet insufficient in calcium for many years may explain this.

m) Tumors

With the exception of carcinoma of the pancreas, which occurs 3–4 times more frequently among diabetics, the incidence of benign and other malignant tumors does not seem to be greater

in diabetics than in nondiabetics of the same age groups. Carcinoma of the endometrium is discussed on p. 776.

8. Marriage, Pregnancy, and Birth

Diabetes mellitus is no reason for staying single. If diabetics marry each other, or someone with a family history of diabetes, they should refrain from having children of their own.

Large babies (birth weight 4.5 kg or more), still births, and neonatal deaths suggest diabetes mellitus, since they are so often observed in women who later develop clinical diabetes, sometimes after 30 years or more.

Before the introduction of insulin, pregnancy was very rare indeed in diabetic women; it usually ended in fatal ketoacidosis. Today, diabetic women's fertility is normal. In our experience at least, the number of spontaneous abortions is not increased, but premature birth and intra-uterine death during the last quarter of pregnancy and neonatal deaths cause an increased loss of babies.

a) Effects of Pregnancy on Diabetes

Pregnancy has a diabetogenic effect, but only in the sense that a potential or latent or subclinical disease may become clinically apparent. This is supported by observations that diabetes is more common in married than in single women, and that its frequency increases with parity. In uniovular diabetic twins, the one with earlier and more pregnancies develops clinical diabetes before her twin sister. Organic hyperinsulinism improves during pregnancy.

Every growth process involves an increased insulin requirement. The higher number of granula, hyperplasia of the B-cells and the raised IRI in the blood during late pregnancy are signs of elevated insulin production. During pregnancy, in nondiabetic women too, the insulin content of the blood is found to be considerably higher in the fasting state and after an intravenous injection of glucose.

Why is the insulin requirement increased during pregnancy? Refer to p. 86 for the diabetogenic properties of growth hormone. The growth hormone produced by the pituitary is not increased during pregnancy; but a hormone with similar chemical and active properties is formed in the placenta (the human placental growth factor) and found in the blood (see Chap. V, p. 84, and Chap. XI, p. 677).

Growth hormone and the glucocorticoids are hormonal antagonists to insulin, which cause hyperglycemia and an increase of the IRI in the serum. Placental tissue also seems capable of binding and breaking down insulin. Insulin is essential for growth, the growth hormone having an anabolic effect only in the presence of insulin (see p. 88).

Probably, the diabetogenic action of pregnancy is due to the unknown placental growth factor and perhaps also to glucocorticoids. This would explain why latent diabetes is often recognized during pregnancy, and why it seems to "disappear" after delivery. However, diabetes is not cured, even if the fasting blood sugar values are normal and the urine contains no sugar. Suitable tolerance tests demonstrate a latent diabetes. Treatment is essential in these cases, as otherwise the diabetes will become overt within a few years. JOHN states that 60% of women showing temporary glycosuria during pregnancy, develop clinical diabetes 1–25 years later.

Pregnancy increases the intensity of preexisting clinical diabetes. Today, the raised insulin requirement can be checked by adaptation of the insulin dosage. The fact that the quantity of insulin often needs little or no adjustment, sometimes even a reduction, can be explained by intensified medical supervision and improved dietary discipline of the patient.

In addition to increasing the insulin requirement, pregnancy can also lead to a *fall in the renal threshold for glucose* and to *lactosuria*. During gestation, there is an increased risk of urinary tract infection and renal failure.

About a quarter of all women show glycosuria during the course of pregnancy (FLYNN). This can be a *renal*, i.e. *normoglycemic glycosuria*, a so-called glycosuria of pregnancy. It is characterized by the constant excretion of abnormal amounts of glucose in the urine, despite normal or subnormal blood-sugar levels in the oral glucose-tolerance tests. Pregnancy leads to a physiological increase of the glomerular filtration rate. If the tubular capacity to reabsorb the filtrated glucose is exceeded, renal glycosuria occurs in healthy subjects as well as in diabetics. After delivery, this glycosuria disappears.

Since MILLER observed 4 still births and 3 overlarge babies in 11 pregnancies with normoglycemic glycosuria and Pomeranze saw a woman with glycosuria of pregnancy who developed clinical diabetes 14 years later, the question has arisen of whether some of these patients, or perhaps all of them, should not be considered potential diabetics. This question is not yet settled, and so far, glycosuria of pregnancy remains suspect of potential diabetes, requiring long-term medical supervison.

A fall in renal threshold may, but does not necessarily, develop during pregnancy in women

with clinical diabetes. In this event, more blood-sugar estimations are needed to control the diabetes. Occasionally, the renal component of glycosuria is so strong that the carbohydrate intake must be increased to avoid ketoacidosis. After delivery, the renal threshold reverts to the previous level.

Lactosuria is found only during the last days of pregnancy and during lactation. It may affect urine tests. Chromatography can reveal small amounts of lactose in the urine even several weeks before delivery. In addition to lactose, galactose is also eliminated in the urine; if large amounts are present, even urine tests based on oxidase methods (e.g. testape), become unreliable.

The tendency to infections of the urinary tract is especially high in pregnant diabetics. They are treated in the same way as in nondiabetics.

The majority of growth-onset diabetics die of renal failure. Pregnancy is very dangerous in women with renal damage, and especially for diabetics with impaired renal function.

Diabetes mellitus as such is no indication for termination of pregnancy, nor even is ketoacidosis, which would only be exacerbated by surgery. Diseases of the kidneys or heart may render the continuation of pregnancy dangerous or impossible in diabetic and nondiabetic women alike. The stress of pregnancy weighs more in a diabetic than in a normal woman. Therefore, the number of pregnancies should be limited to 2, in favorable circumstances to 3 in diabetic women.

The *treatment of diabetes during pregnancy* is based on a diet of 180–250 g of carbohydrates and 1.5–2 g of protein per kg body weight, with so little fat that the weight increase remains within the physiological limits, i.e. at the beginning 1 kg per month, and 1.5 kg per month during the last trimester. Table salt is reduced or omitted in case of water retention. Acetonuria is treated by an increase in carbohydrates and reduction of fats in the diet and if necessary an augmentation of insulin.

In early diabetes, when diet and exercise alone are insufficient to control the diabetes, insulin is used. During the first 3 months of pregnancy, insulin is adjusted cautiously to avoid hypoglycemia. Later we try to keep the blood sugar as close as possible to normal limits. Oral hypoglycemic drugs are eliminated (see p. 783).

Signs of a *late toxicosis* (preeclampsia) must be treated by bed rest, a salt-free diet, and when necessary, hypotensive drugs and salidiuretics. It may sometimes be necessary to increase the dosage of insulin.

As soon as delivery is imminent, the daily or twice-daily insulin injections are replaced by 3 injections of soluble insulin. If labor starts while the patient is still under the influence of long-acting insulin, an intravenous dose of fructose must be substituted for the missed meal.

There is usually a pronounced but temporary decrease in the insulin requirement immediately after delivery, whether delivery is vaginal or by cesarian section. This seems to be due to the loss of the placental growth factor.

b) The Influence of Diabetes on Pregnancy

Before the insulin era, the *mortality rate of the mothers* was as high as 45% (GILBERT and DUNLOP), whereas today, the lowest mortality figures are 0.3–0.7%, in comparison to those of 0.32–0.37 for nondiabetic women.

According to WHITE, only one in three untreated diabetics has a chance of producing a live child. The *mortality rate of the children* largely depends on the experience, knowledge and cooperation of the team consisting of diabetologist, obstetrician, anesthesist and pediatrician.

The course of the pregnancy to the 30th week is usually normal, with the exception of the susceptibility to infections. Hyperemesis in the early stages of pregnancy does not appear to be particularly severe or frequent. It usually requires adaptation of insulin to the irregular food intake.

The main obstetric difficulties occurring during the last 6–9 weeks are:

1. increased tendency to hydramnios;
2. increased tendency to toxemia;
3. macrosomia or microsomia of immature infants;
4. intra-uterine death (RUST).

The frequency of *hydramnios* in normal pregnancy is estimated at 0.5–1%, in diabetics at 19%. As mentioned above, WORM and SIEGELER showed how hydramnios can be avoided by adequate insulin therapy.

The frequency of toxemia in nondiabetic women is reported to be about 10%; in diabetics, according to the literature, it is 8–48%. Prevention, i.e. avoid of abnormal increase in weight, seems very important. A gain of 400–500 g per week suggests toxemia and calls for admission to hospital. If bed rest does not lead to increased urine volume and to weight loss, diuretics are indicated, even if they increase the requirement of insulin still further.

Nephropathy present before pregnancy considerably increases the risk of toxemia. The infants of diabetic mothers with nephropathy

are small whilst those of diabetic mothers with good renal function are generally considerably larger and heavier, but more immature than children of healthy women with the same duration of pregnancy.

The most frequent and the most deceptive complication is intra-uterine death. According to HAGBARD, this occurs before the 35th week in 3% of cases, but the risk increases with every week and reaches 25% after the 37th week. Neonatal mortality, however, decreases from 19% in infants delivered between the 33rd and 34th week to 11% with delivery during the 35th and 36th week. *Placental insufficiency* is considered the main cause of intra-uterine death.

If fetal movements decrease in intensity and frequency this is a sign for alarm. If the time intervals between the fetal activity felt by the mother grow longer, the life of the child seems to be in danger. Bed rest for the mother, if necessary combined with inhalations of oxygen, up to 8 liter/min for 10–15 min, 2–8 times daily, seem to help the fetus, but this is a clinical impression only and we do not know the mode of action of oxygen therapy.

Some indication of placental function is obtained by measuring urinary estriol elimination. If it falls below 8 mg/24 h during the 35th week or later, the life of the fetus is in danger; according to KYLE, values below 4 mg were always followed by fetal death within 48 h.

The value of ovarian hormone therapy is controversial. We have never used the doses indicated by WHITE, because the effectiveness of this treatment has not been proven and damage to the fetus not definitely excluded. If the estrial excretion diminishes, we give 40 mg of estrogen daily per os and up to 250 mg hydroxyprogesterone i.m.

Choosing the optimal time and the best mode of delivery is the main obstetric problem in diabetic women, i.e. the time when the risk of postnatal immaturity of the newborn is about equal to that of intra-uterine death. Before the 35th week, the chances for neonatal survival are small, after the 38th week, intra-uterine death becomes frequent.

The following factors should be taken into consideration in determination of the time of delivery:

1. obstetric history of the mother;
2. the evolution of the present pregnancy in regard to complications and diabetes. A sudden drop in the insulin requirement during the last weeks, for instance, may indicate impending intra-uterine death;
3. comparison of the risk of intra-uterine death against postnatal prematurity. To evaluate this, fetal movements, excretion of estriol in the urine examination of the amniotic fluid and changes in insulin requirement give valuable hints.

The most frequent time of delivery is three weeks before the normal term. We allow young women whose diabetes has been discovered during the course of the current pregnancy, who have never before had any perinatal loss and whose pregnancy is progressing normally to go on to term, but never past the 40th week. However, an obstetric history with one or more stillbirths calls for induction of labor before this, as does diabetes with vascular damage, since these women are obstetrically older than their age and therefore more prone to fetal loss.

The determination of the duration of the pregnancy is much more reliable in women who know the date of conception exactly, because they have recorded their morning rectal temperature regularly. In such cases, we consider 268 days the normal duration of pregnancy.

An indication of sufficient maturity of the fetus is given by the appearance of the distal femoral ossification centers on X-ray examination. If possible, we postpone the delivery until they are visible, usually 3–4 weeks before term. We have never lost a newborn baby delivered after the ossification centers had become recognizable.

Normal delivery, if proceeding without complications, involves the least stress for both mother and child. Cesarean section involves the risks of any major surgical procedure, which have been reduced considerably by experience and modern anesthesia. The uncertainty of prognosis for delivery per vaginam increases with the severity and duration of the diabetes and with the prematurity of the infant, whilst the duration of cesarean section and the stress involved are known beforehand.

We consider a cesarean section indicated:

1. if the mother has previously lost a child before or during labor;
2. if the relative positions of head and pelvis are unfavorable or the position of the fetus is abnormal;
3. in primiparae aged 28 or ever;
4. if rapid delivery is urgent;
5. if delivery is necessary 4–5 weeks before term or earlier. Table 2 summarizes our personal experience.

Early vaginal delivery is started by oxytocin drip, often combined with surgical rupture of the membranes. Delivery should be completed within 12 hours.

Before cesarean section, the only premedication is atropin. No opiates or barbiturates are given. General anesthesia is started by Pentothal (penthiobarbital sodium); after delivery of the

child, usually 6–8 min after the beginning of general anesthesia, we give nitrous oxide and oxygen.

The *puerperium* is the same in diabetic as in nondiabetic women. Breast-feeding is more difficult for the former.

c) Influence of Maternal Diabetes on Fetus

Diabetic embryopathy must be differentiated from diabetic fetopathy in the children of diabetic mothers.

Malformations resulting from diabetic embryopathy are three times more frequent than similar malformations in the general population. The incidence of fetal malformations is 6 times higher. The rate of congenital abnormalities in our own observations was 12.8%. They are not uniform in character. Those affecting the heart seem to be preponderant. We have seen several cases of deformations of the ear lobes and preauricular appendices, which, in two children, were combined with a congenital facial paralysis. The teratogenic agent of the embryopathia diabetica is unknown; we do not know whether too much or too little sugar is of any importance.

The frequency of intra-uterine death in children of diabetic mothers during the last weeks of pregnancy and the increased occurrence of stillbirths in the same family underline the importance of the fetopathia diabetica. If such children are delivered in a macerated state, pathologico-anatomical investigations yield little information. Often children may be born alive, but have little resistance and die within a few hours or during the first two days. Some are born in a condition resembling hydrops congenitus, they are swollen, flaccid, adynamic, cyanotic, dyspneic and hepatomegalic. Another group, in which prognosis is considerably better, shows macrosomia with a cushingoid hydropic appearance, which may be seen also in macrosomia with normal or even subnormal weight. These newborn infants have a tomato-coloured bluish red and thick face with flaccid cheeks and double chin; the neck is hidden by an accumulation of adipose tissue on chin and chest; hair is highly developed, covering the skull like fur extending down to the frontal and temporal regions.

Some authors attribute the macrosomia to maternal hyperglycemia. During the last weeks of pregnancy, fetal blood sugar is only a little lower than the maternal value. The high blood sugar of the fetus stimulates the β-cells of the pancreas, thus increasing their number and insulin production. This leads to an increased formation of glycogen in the liver and in the musculature and enhances fat and protein synthesis. There may be other factors contributing to macrosomia, such as adrenal activity. In a child who died 3 hours after delivery, WILLI observed suprarenal glands which were almost the size of the kidneys and weighed 17 g. This perhaps explains the Cushing-like appearance. However, the hypertrophy of the adrenal glands may also be a consequence of hyperglycemia.

Only a minority of the children of diabetic mothers are exempt from macrosomia. The birth of a small baby to a diabetic mother may indicate maternal nephropathy.

Immaturity and decreased vitality are typical of children of diabetic mothers. They are more immature than their weight and the duration of pregnancy would suggest. The discrepancy between anatomical oversize and functional weakness is a striking feature of macrosomia (WILLI, 1965).

Erythroblastosis in the blood, and extramedullary hematopoiesis in various organs, mainly in liver, spleen, lymph glands and kidneys, are typical signs of immaturity. The same is true of the delay in the development of ossification centers.

Immatury and reduced vitality make adaptation to extra-uterine life more difficult. Birth injuries are more frequent on account of immaturity and macrosomia.

The respiratory distress syndrome, due to the formation of *hyaline membranes*, is a relatively common cause of postnatal death. Its pathogenesis has not yet been fully elucidated. It is always accompanied by respiratory and metabolic acidosis. A pH value of less than 7.15 is an ominous sign.

During the first 6 hours after delivery, the blood sugar of the newborn falls, sometimes to as little as 20 mg%, without causing hypoglycemic symptoms. In spite of glucose administration, the blood sugar of these premature infants may remain below 40 mg%.

Another remarkable feature is the fall of the blood calcium level which frequently reaches pathological levels 6–8 hours after delivery, thus causing hypocalcemic tetany; this, however, generally remains latent. It is recognizable clinically by tremor and excessive mechanical irritability of the peripheral nerves.

About one third of our children develop *hyperbilirubinemia* of over 15 mg%, which is due to the inability of the immature liver to conjugate bilirubin during the first days of extra-uterine life. In contrast to morbus hemolyticus, hyperbilirubinemia reaches its maximum only after 3–4 days.

During the first day, edema may develop, although the newborns urinate abundantly,

lose weight and take little fluid. Apparently, much water moves from the intracellular to the extracellular space, causing hypervolemia, a great strain on the heart which may show considerable dilatation. All these difficulties during the change from intra- to extra-uterine life are transient and generally subside within 8 days.

Treatment of Newborn Infants of Diabetic Mothers. Immediately after delivery, whilst the umbilical cord is tied, the head is kept low and the body wrapped in warm towels; nose, mouth, throat and upper respiratory tract are vigorously aspirated. After a cesarean section, the stomach must be emptied. In case of cyanosis, oxygen is administered. Then the infant is placed naked in an incubator and treated like a premature baby. However, this is not necessary for children who are mature or have been delivered only 1–2 weeks before term.

If no respiratory distress syndrome develops, the infant is taken out of the incubator 12–24 hours later. If cyanosis and dyspnea increase, oxygen administration is increased to 30%, maximum 40%, but never for longer than is absolutely necessary, since too much oxygen may favor the formation of hyaline membranes. The humidity of the air in the incubator is increased to 90–100%, because oxygen therapy leads to exsiccation of the mucous membranes.

The presence of severe metabolic acidosis (base excess more than −10 mEq) demands i.v. injection of sodium bicarbonate in 10% glucose and repeated checks of pH and base excess in the arterial blood. In very severe cases, prolonged and controlled artificial respiration may prove lifesaving.

General conditions permitting, food intake begins at the latest 8 hours after delivery; it is delayed in case of respiratory distress syndrome. At first, the infants receive 5% glucose orally, 10 ml 5 times during the first 12 hours, and later breast milk. Early and abundant glucose intake is believed to prevent severe hyperbilirubinemia and metabolic acidosis.

If hyperbilirubinemia exceeds 20 mg%, exchange transfusion is necessary.

Hypocalcemic cramps are treated by i.m. injections of 3–5 ml of 10% calcium gluconate, acute *dilatation of the heart* by i.m. injection of 0.5 ml Cedilanid 1–3 times, or oral administration of 4 drops 3 times, during the first day, later on 2 drops 3 times daily.

9. Infantile and Juvenile Diabetes Mellitus

Diabetes mellitus which develops in the still immature organism, is characterized by its tendency to develop ketoacidosis and by the lability of blood sugar. It easily leads to hepatomegaly, retarded growth, and the relatively early appearance of the long-term diabetes syndrome. Gastrointestinal disorders and infections are quite common at this age and cause significant variations in carbohydrate tolerance. It is surprising how easily children can adjust themselves to the necessities of the disease. Difficulties most commonly start at puberty.

In contrast to *neonatal glycosuria*, *congenital diabetes* is very rare. It is claimed that 80% of newborn infants show glycosuria before the first feed (see p. 774). Towards the end of the second week, in premature babies a little later, neonatal glycosuria disappears.

Clinical diabetes may appear at any age. Periods of rapid growth between the 5th and 7th, and the 10th and 14th year are preferred. Girls develop the disease rather earlier than boys.

After an infectious disease or an immunization, but usually quite unexpectedly, children lose their appetite, start to wet their beds again or develop severe constipation, symptoms followed within a few days by polyuria, polydipsia, weakness, weight loss and the signs of precoma.

Temporary glycosuria, postprandial hyperglycemia and abnormal glucose tolerance tests can be detected months or even years in advance. The renal threshold for glucose is often decreased, while the fasting blood sugar may remain normal for a long time. The diagnosis must therefore be made with the aid of *blood sugar determinations after meals.*

According to their height, their formation of ossification centers and their dental development, the majority of diabetic children show advanced growth compared to normal ones at the time clinical diabetes is discovered. As the illness progresses, this situation is reversed, unless early good control assures continuation of growth.

Without treatment, the majority of growth-onset diabetics die within a few days to weeks from ketoacidosis. When treatment is started early, the diabetes is usually successfully under control within a few weeks with the help of diet and insulin. The following improvement of tolerance sometimes enables patients to maintain normal blood sugar values with diet alone. However, this remission does not occur in all the cases and lasts only a few weeks or months, rarely years, before the carbohydrate tolerance deteriorates again. Insulin therapy must then start again, this time permanently. It is not yet possible to state definitively whether oral hypoglycemic agents will prolong the remission.

As in the adult, treatment includes diet, insulin, adjustment in the way of living, and the

education of the patient and his relatives. Useful as a low calory diet is in the adult, it is detrimental in children. During childhood, the diet should not be adapted to the lowest possible dose of insulin, but rather to normal growth and well-being, and the prevention of diabetic angiopathy.

The diet differs in four principal points from that of healthy children of the same age groups:

1. The carbohydrate and protein contents must be about constant, but adapted to age.
2. Foods with a high concentration of carbohydrates, such as sugar, sweetened foods, and drinks, honey and grapes, are generally excluded (exceptions see below).
3. If possible, proteins are given in addition to the carbohydrates with the three main meals, so as to slow down the absorption of the latter.
4. The protein content is higher than for the healthy child, since we cannot avoid a loss of nitrogen through the urine.

We discard the "free or normal diet", since it considerably reduces life expectancy! The statement that irregular intake of food can be compensated for by constant adjustment of the insulin dosage, has not yet been proved in unhospitalized children. In our experience it is not correct and endangers the children concerned. The feeds of even healthy babies are specified and the diabetic babies are continued on normal feeds, but extra sugar and malt products are omitted. Rules for feeding for the first 6 months (WILLI and KOLLER):

Milk: 1/10 body weight, maximum 600 g = 3 milk equivalents.

Carbohydrates: 1% of body weight, rounded off to 5 or 10 g, given as gruel or farinaceous foods (a child weighing 4200 g receives 45 g carbohydrates = $4\frac{1}{2}$ bread equivalents).

From the 2nd to 3rd month, 1–3 ounces of fruit juice are given in addition, the nutritional value of which is not taken into account. Later, vegetables are also added, in small harmless quantities to begin with. As soon as potatoes are introduced, usually from the 5th month, these are measured in bread equivalents (see Sect. 7b).

The nutritional requirements of children and juveniles vary greatly from one child to another and for the same child, depending on whether he is in a phase of intensive growth and on his physical activity. On account of this, no absolute values for carbohydrates, fats, or proteins can be given. The dietary regimen summarized in Table 26 for diabetic children and juveniles, is used as "basic diet". We begin treatment with this diet and it is continually adjusted to the individual needs and the development of the child.

In diabetic children who are not overweight, we leave the fat allowance open. Fats are used to meet the caloric requirements which, at this age, vary considerably from hour to hour.

Any attempt to treat a diabetic child in the beginning without insulin or with sulfonylurea derivates carries the risk of losing the reserves of endogenous insulin and of approaching the stage of complete islet insufficiency.

Diabetic dwarfism is characterized by a retardation of more than 10 cm below average height, and by the infantile psyche in the presence

Table 26. Basic diet for diabetic children and juveniles

		1–3 yrs.	4–6 yrs.	7–9 yrs.	10–12 yrs.	13–15 yrs.	16–19 yrs.
1. Breakfast	Bread equivalents	2	2	4	5	5	5
	Milk equivalents	1	1	1	1	1	1
	Protein equivalents	–	–	$^1/_2$	1	1	1
2. Morning snack	Fruit equivalents	–	1	2	2	2	2
3. Lunch	Bread equivalents	2	2	2	2	2 (3)	2 (3)
	Vegetable equivalents	$^1/_2$	$^1/_2$	1	1	1	1
	Protein equivalents	$^1/_2$	$^1/_2$	1	$1^1/_2$	2	2
	Fruit equivalents	1	2	2	2	2	2
4. Tea	Bread equivalents	1	2	4	4	4 (5)	4 (5)
	Milk equivalents	1	$^1/_2$	1	1	1	1
	Fruit equivalents	1	2	–	–	–	–
5. Dinner	Bread equivalents	2	2	2	2	2 (3)	2 (3)
	Milk equivalents	1	$^1/_2$	–	–	–	–
	Vegetable equivalents	$^1/_2$	$^1/_2$	1	1	1	1
	Protein equivalents	–	$^1/_2$	1	1	1	1 (2)
	Fruit equivalents	1	2	2	2	2	2
Approx. content of	Carbohydrates	140	180	220	230	230 (260)	230 (260)
	Proteins	45	60	75	85	90 (95)	90 (100)

() = Values for boys.

of normal intelligence. The moon-shaped face is explained by thickening of the skin. The abdomen is unusually large, the ossification centers develop late, and sexual maturation is often delayed. In the early stages, the size of the liver varies. Later, it becomes quite large and its anterior border is felt below the umbilicus (Mauriac syndrome). There is no splenomegaly. The abdominal cutaneous veins are distended. Diabetic dwarfism is probably due to a wrong or deficient diet and to inadequate administration of insulin. The Mauriac syndrome has become much less common in recent years.

The intelligence of diabetic children used to be labeled as above average. Whether this is true is difficult to decide, since unintelligent diabetic children die earlier than gifted ones.

10. Surgical Procedures in Diabetics

Today, diabetes mellitus is no longer a reason for refraining from an essential operation; it is at most a reason for postponing surgical intervention. However, it is a technical mistake to operate on a diabetic without treating the diabetes pre- and postoperatively.

An operation makes preexisting diabetes worse and may provoke ketoacidosis. On the other hand, uncontrolled diabetes increases the risk of operation due to its tendency to hemorrhage, delayed healing and infection.

The preoperative treatment is aimed at increasing resistance by eliminating dehydration, by replenishing the glycogen stores, by preventing acidosis, and by lowering the blood-sugar concentration to normal or almost normal values. The insulin requirement and the insulin sensitivity should, if possible, be established before the patient undergoes surgery.

Whenever feasible, the diabetes must be controlled before an operation. If surgery is urgent, intravenous injections of soluble insulin every half to one hour will permit sufficient control over diabetes and dehydration in a few hours. Complications during and after surgery are less frequent when there is sufficient time to eliminate ketoacidosis, to reduce the blood sugar to below 160 mg per cent before meals, not only before breakfast, and the urine sugar to below 10 g per 24 hours. Cooperation between surgeon, diabetologist and anesthetist is important.

The diet should contain relatively ample amounts of carbohydrates (180 to 250 g carbohydrates for an adult). Preoperative undernourishment must be avoided, since it increases the tendency to acidosis and depletes glycogen depots.

Treatment with hypoglycemic tablets is only continued if control of diabetes is perfect and food intake unchanged by the operation, e.g. cataract operation. Treatment with insulin is preferable for all other cases, since the surgical stress increases the insulin requirement to an unpredictable extent. Long-acting insulins are replaced by soluble insulin. Three daily examinations of the urine, as described on p. 787 permit the insulin dosage to be adjusted to the existing metabolic situation. Blood-sugar measurements before meals prevent overdosage of insulin.

Prolonged fasting must be avoided by parenteral administration of carbohydrates.

During an *emergency operation*, which does not permit proper preparation of the patient, intravenous administration of whole blood or of 5% fructose is started and soluble insulin added to the tube of the infusion every $\frac{1}{2}$–1–2 hours, according to the blood-sugar level determined by a rapid method every hour before and during the operation, and every two hours after it. During anesthesia, hypoglycemia should be avoided at all costs.

Operations should be postponed in case of ketoacidosis, with the exception of draining an abscess.

Modern general anesthesia is the same for diabetics and non-diabetics. If adrenaline is combined with local anesthetics, the insulin dosage must be adjusted accordingly. No local anesthesia is permitted on feet or toes of diabetics with peripheral angiopathy.

During the operation, hypoglycemia is more dangerous than hyperglycemia.

A solution of 40% dextrose and of glucagon should always be ready during anesthesia of a diabetic patient.

In order to assure normal healing of wounds, the post-operative treatment tries to prevent acidosis and hyperglycemia, but also hypoglycemia with its strain on the myocardium. In older patients, e.g. after prostatectomy, normalization of blood sugar may lead to oliguria which will disappear when we permit a small rise of the blood-sugar level.

As long as the patient cannot drink or eat, we give 1–$1\frac{1}{2}$–2 liters of 5% fructose or 2.5% fructose and 2.5% glucose, or 2 parts of physiological glucose and 1 part of physiological saline solution by intravenous drip.

When fluid intake per mouth is permitted, we first give tea with 1–2 teaspoons of fructose per glass of tea.

With the help of 2–4 blood sugar determinations per day, the insulin dose is adjusted to the need, until the patient can resume his ordinary diet.

Draining of an abscess is usually followed by a sudden reduction of the insulin requirement,

permitting reduction of the insulin dosage. Hypoglycemia must be avoided during operations on the eye, since it causes an increase in the intraocular pressure.

11. Special Forms of Diabetes Mellitus

a) Diabetes Mellitus after Pancreatic Failure

α) After Pancreatectomy

The operation only results in diabetes mellitus if at least 90% of the pancreas is removed. Carcinoma of the pancreas, organic hyperinsulinism and chronic recurrent pancreatitis are indications for pancreatectomy. The subsequent diabetes needs remarkably little insulin for its control, only 10–50 units per day. This may be partly explained by the simultaneous loss of the exocrine enzymes of the pancreas and of glucagon. Retinopathy was found in a few patients who survived the operation long enough.

β) After Pancreatitis

Hyperglycemia and glycosuria which are present during the course of acute pancreatitis almost always disappear after the inflammation has subsided (see p. 798). On the other hand, chronic pancreatitis, especially in the recurring form, leads to progressive destruction of the exo- and endocrine tissue and finally to calcification.

This type of diabetes is characterized by its fluctuations. The insulin requirement increases considerably during painful phases and falls during remission. The terminal malabsorption syndrome fakes "improvement" of the diabetes.

This form of diabetes is often followed by neuropathy, later retinopathy, whilst nephropathy is rather rare.

γ) Due to Hemochromatosis

Hemochromatosis damages the islet tissue of the pancreas, and thus causes diabetes mellitus. We distinguish *primary, idiopathic, inherited hemochromatosis* from *secondary hemochromatosis*, and *hemosiderosis*, which is found in alcoholic liver cirrhosis, in blood diseases after repeated blood transfusions, in rare liver diseases, in porto-caval anastomoses, in inanition, after excessive intake of iron preparations and in protein deficiency of Bantu Africans.

Idiopathic hemochromatosis is now looked upon as a hereditary diseases with dominant inheritance. It is often met in the latent form, i.e. without tissue changes, in other members of the family. The disturbed regulation of iron absorption leads gradually to an excessive accumulation of iron in the tissues. It is 10–20 times less common in women (menstruation!) than in men. The predominant clinical features are the grey-brown pigmentation of the skin (bronze diabetes), liver cirrhosis, diabetes mellitus, cardiac failure, and in the male hypogonadism (see 799).

The diabetes mellitus develops relatively late. In the early phases, it is usually mild, later more severe, and sometimes even very severe. There is a tendency to acidosis, but the diabetes can unexpectedly become latent again. The blood sugar is labile and the insulin acts in spurts, so that severe hypoglycemic shock may arise, which must be treated by the intravenous administration of dextrose. On the other hand, resistance to insulin has been reported relatively often. The long-term diabetic syndrome has also been observed in hemochromatosis, but less frequently than in the usual form of diabetes mellitus, on account of the short duration of diabetes, the rarity of hypertension, hypercholesterolemia and obesity, and due to moderate or poor nutritional status as well as to pituitary hypofunction.

The *diagnosis* of hemochromatosis is simple in the presence of all the clinical features. In the early stages, when one or two of three typical symptoms are missing or not yet evident, the diagnosis may be difficult and sometimes it is not possible until a post-mortem examinations is performed.

The diagnosis can be confirmed by the following methods: estimation of the iron and copper contents of the blood, measurement of the iron-binding capacity of the blood, the histological examination of the skin or, more reliably, of the liver tissue, and further presence of hemosiderin in the sternal puncture and in the urinary sediment. The last two symptoms are also present in hemosiderosis.

The prognosis of hemochromatosis has improved since insulin has been introduced, combined with phlebotomies.

The most common *causes of death* are hepatic insufficiency, hemorrhage from esophageal varicose veins, diabetic coma, primary carcinoma of the liver, cardiac failure or intercurrent infections, seldom a severe state of shock associated with flooding of the organism with ferritin.

The *therapy* aims at delaying the development of cirrhosis of the liver, at compensating the diabetes, and, above all, at removing iron from the body.

The *diet* should be relatively high in proteins, vitamins and carbohydrates, but low in fat. Wine must be avoided!

There are two ways of *eliminating the iron* – repeated phlebotomy or desferrioxamine B. The former is the treatment for primary hemochromatosis, since a large amount of iron is withdrawn rapidly in this way (2 liters of blood contain approx. 1 g of iron). Desferrioxamine B (Desferal), on the other hand, would, at the most, be considered in cases with anemia.

The indications for treatment with insulin are similar to those for the ordinary form of diabetes mellitus, but it may be necessary to give the insulin intramuscularly due to poor absorption from subcutaneous tissue. Sulfonylurea derivatives appear to have no effect, whereas biguanides sometimes reduce the blood sugar.

δ) Due to Fibrosis of the Pancreas

Mucoviscidosis or *fibrocystic disease* of the pancreas is an inherited familial disease, in which the function of all the exocrine glands is disturbed, especially those producing mucus. The disease often results in chronic bronchitis, bronchiectasis, and finally in chronic pulmonary insufficiency. In children meconium ileus, cirrhosis of the liver with congestion of the portal veins and prolapse of the rectum are common concomitant features. Insufficiency of the exocrine pancreatic glands can occur alone or combined with disturbances of the respiratory tract. It may lead to a malabsorption syndrome in spite of the increased appetite and ample food intake. The sodium and chloride contents of sweat, and to a lesser extent of saliva and tears, are definitely increased. The activity of the pancreatic ferments in the duodenal juice and in the feces is reduced. Recently, a glycoprotein which may be characteristic for the condition has been demonstrated in the mucus, in the epithelial cells of the mucous glands and in the stool. The progressive fibrosis of the pancreas can destroy the islets and consequently lead to labile diabetes mellitus in its late stages.

b) Diabetes Mellitus Resulting from Disorders of Other Endocrine Glands and from Hormone Therapy

α) Pituitary

See p. 113 about diabetes in acromegaly.

β) Adrenal Glands

See p. 335 about diabetes in primary aldosteronism, p. 432 in pheochromocytoma, and p. 349 in Cushing's syndrome.

γ) Thyroid Gland

It is not known to what extent hyperthyroidism can produce diabetes. It can give rise to oxyhyperglycemia, and it makes preexisting diabetes worse. Hyperthyroidism must be considered in every case of acidosis without obvious cause and in every case of insulin resistence. Correction of the hyperthyroidism improves associated diabetes mellitus, but does not cure it. Refer to p. 187 for more details.

c) Diabetes due to Deficiency of Adipose Tissue

Lipoatrophic diabetes (LAWRENCE), *the diffuse, diabetogenic lipoatrophy*, or *lepercaunismus* is very rare and affects predominantly young people. The loss of fatty tissues of the skin and internal organs makes the conversion of carbohydrates into fats impossible and deprives the insulin of one of its end-organs. It leads to an increase in growth, which, however, in contrast to acromegaly, stops with puberty. The diabetes mellitus becomes overt when growth ceases and there is a fall in the protein requirements. The inability to deposit fats in the adipose tissue explains the hyperlipemia and the liver steatosis. The "fat-mobilizing" substance recently found in the urine will probably explain lipoatrophy and diabetes mellitus, but this still awaits further confirmation (HAMWI).

The characteristic clinical features of the disease are disappearance of the adipose tissue, hyperlipemia, hepatomegaly, and an elevated basal metabolic rate, as long as this is calculated in the usual way according to the body surface and not to the total body weight. Splenomegaly is not very uncommon, and it has been observed several times in combination with *acanthosis nigricans*. Children have a strongly developed musculature (Hercules boys), large prominences, and crinkly hair which gives them an elf-like appearance (leprecauns). Insulin and growth hormone in the blood are normal. This form of diabetes has no tendency to ketoacidosis and is resistent to insulin, perhaps since one of its main sites of action is missing and/or the excessive mobilization of fatty acids inhibits the action of the islet hormone. A low-fat diet causes a regression of hyperlipemia and of diabetes.

The relation to *progressive lipodystrophy* (BARAQUER-SIMONS) is not yet clear. This may be an early stage of diffuse diabetic lipoatrophy. In contrast to the latter illness, the disappearance of adipose tissue begins in the face and spreads gradually over the upper part of the body. It is often combined with diabetes, which sometimes proves resistent to insulin, and with hepa-

tomegaly. Progressive lipodystrophy is much more common in women than in men.

d) Diabetes in the Prader-Labhart-Willi Syndrome – Myatonic Diabetes ("Flour-Bag Dwarf")

In 1956 PRADER and his team described 9 children with obesity, acromicria, hypogonadism and myatonia in the early years of life, associated with retardation in mental and physical development, and also with disorders of carbohydrate metabolism. In the newborn, myatonia was predominant, and later obesity and oligophrenia. In boys, hypogonadism was the prevailing clinical feature. The myatonia was obvious from birth since the movements of the infants were absent or very weak.

The muscular hypotonia decreases somewhat after birth, so that the children can walk from the second year on wards. The obesity usually develops during the first year, the abdomen being particularly involved. The acromicria and the short neck, knock knees, and pallor of the skin all combine to give the appearance of a flour bag. The hypogonadism – small, ectopic or absent testicles with a rudimentary scrotum – leads to delayed and incomplete puberty.

In the first years of life, there is hypersensitivity to exogenous insulin, and later a reduction in carbohydrate tolerance. Finally, a form of diabetes develops, which differs from the usual infantile diabetes in the absence of ketosis and the good response to dietary treatment and sulfonylurea derivatives. A severe case of this form of diabetes can be considerably improved by the reduction of overweight, which is often enormous. With the exception of diabetes, the syndrome appears to be the exact opposite of lipoatrophy.

The nature of the disorder is unknown. Familial occurrence indicates an inherited disease, and the advanced age of the mother at the time of the patient's birth suggests a chromosomal anomaly in the baby.

e) Young Diabetics Independent of Insulin

In Europe, juvenile diabetes which can be controlled for decades by diet alone or in combination with oral hypoglycemic agents, and does not need insulin even during pregnancy is very rare indeed.

In Natal, there appear to be many young overweight diabetics among the Indians who do not need insulin (CAMPBELL). These observations stem entirely from developing countries, where the nutritional conditions are difficult to assess. On the other hand, this form of diabetes may have a different pathophysiology. The type of diabetes in Natal Indians differs from the K-type described by SHAPER in Campala, Uganda. In the latter type, the diabetes becomes overt between the 16th and 35th year and in spite of the thinness and the typical classic beginning of the metabolic disorder, the patients seem able to manage without insulin. In contrast to the K-type, there is the J-type described by HUGH-JONES among young underweight Africans in Jamaica. This type necessitates large amounts of insulin, but the patients show only slight ketotic tendencies. Such cases have also been described in other warm countries. The J-type could be explained by undernourishment and deficiency of animal proteins and of fats.

F. Diagnosis of Diabetes Mellitus

E. R. FROESCH

The correct diagnosis of diabetes mellitus is of utmost importance to everyone and so are the consequences. In spite of the frequency of the disease, criteria for the diagnosis of diabetes mellitus are still poorly defined. The reasons for this unsatisfactory state of affairs are multiple:

1. Glucose tolerance decreases with age, so that the criteria for a normal glucose tolerance curve vary for each age group.

2. The so-called "normal" curves are probably subject to an inherent error. The group of so-called normal control subjects undoubtedly contains some individuals with potential diabetes.

3. In U.S. and in England venous blood is usually used for estimating blood sugar, whereas in Europe capillary blood, the glucose content of which corresponds to that of arterial blood, is mostly used. In the fasting state the arteriovenous difference of blood glucose is very small, but during the glucose tolerance test it may rise up to 60 mg%. One hour after ingestion of 50 g of glucose orally, this difference is 30 mg% on the average.

4. Glucose tolerance is altered by the following factors: severe, acute illness, physical inactivity or rest, prolonged fasting. A normal diet with at least 200 g of carbohydrates for three days should precede a glucose tolerance test. Glucose tolerance decreases during pregnancy. About 50% of all patients with cirrhosis of the liver have a "diabetic" glucose-tolerance curve. The majority of syndromes of endocrine hyperactivity are associated with carbohydrate intolerance. Hypokalemia of any etiology causes

glucose intolerance. The following commonly used drugs also decrease glucose tolerance: glucocorticoids, diuretics and contraceptive drugs.

Polyuria and polydipsia are usually the first symptoms of diabetes mellitus. Examination of the urine for sugar, therefore, is the first diagnostic test for diabetes.

1. Urine Examination for Glucose

Quantitative or semi-quantitative estimations of urinary glucose are essential for the treatment and control of diabetes. For the diagnosis, however, specificity is more important than quantitation.

a) Glucose Oxidase Methods (Tes-Tape, Clinistix)

These tests are based on the specific oxidation of glucose to gluconic acid by the enzyme glucose oxidase. The indicator paper is impregnated with glucose oxidase, peroxidase and orthotolidine (indicator dyes). Glucose oxidase reacts with glucose and oxygen to form gluconic acid and hydrogen peroxide. The latter converts orthotolidine into a blue indicator. The paper strips are dipped into the urine and the colour reaction is read after one minute. If there is no blue coloration, the test is considered negative. Concentrations of glucose up to 0.01% can be detected with glucose oxidase test strips. Normal glucose excretion may reach values of up to 200 mg per day or 15 mg%. A slightly positive reaction with glucose oxidase does not necessarily imply the diagnosis of diabetes mellitus. In such a case, a semi-quantitative test such as a clinitest must be carried out, from which more quantitative conclusions can be drawn about the amount of glucose present in the urine. Samples of urine voided after a meal are suited for detection campaigns for diabetics, since they contain glucose also in cases of latent diabetes, whereas the urine obtained in the fasting state is often devoid of glucose. Apart from this, these specific and easily handled glucose oxidase strips are very well suited for the occasional control of urine in old people with "mild" diabetes.

b) Benedict's Semi-Quantitative Test

Benedict's solution contains sodium bicarbonate, sodium citrate and copper sulfate. Copper sulfate is reduced by glucose under the influence of heating and a blue color develops. Eight drops of urine are mixed with 5 ml of qualitative Benedict's solution in a test tube. The mixture is then kept boiling over a flame for 3 min, or heated in a boiling water bath for 6 min. The color changes give a fairly exact measurement of the sugar present in the urine: blue: no sugar; green clear: 0.1%; green cloudy: 0.3%; green with a yellow sediment: 0.5–1%; yellow: 1%; orange: 2%, red to brown: over 2%. Benedict's test is less specific than the glucose oxidase methods. All pentoses, hexoses and both disaccharides maltose and lactose, also give a positive Benedict's test. When the Benedict's test is positive and the glucose oxidase test strip negative, the sugar present in the urine must be identified by means of paper chromatography.

c) Clinitest, the Tablet Modification of Benedict's Test

Clinitest tablets contain the same ingredients as Benedict's solution plus sodium hydroxide. 5 drops of urine and 10 drops of water are mixed with one tablet in a test tube. The sodium hydroxide present in the tablet causes the mixture to boil. The solution can be shaken 15 sec after boiling has ceased and the reaction read off from a color scale. This test is preferred to Benedict's test by most patients since it is much easier to carry out wherever the patient happens to be. The tablets are hygroscopic and must, therefore, be stored in a dry place. The cost of the tablets is considerably higher than that of Benedict's solution.

d) Quantitative, Polarimetric Examination of Urine Sugar

Naturally occurring D-glucose deflects the plane of polarized light to the right, fructose, protein and beta-oxybutyric acid to the left. Provided that no other optically active substance is present in the urine, the glucose content can be quantitatively measured. During the phase of the stabilization of the blood sugar this method is very useful in hospitalized diabetics, provided that false results due to the substance mentioned above and interfering drugs are excluded.

2. Qualitative Estimation of Ketone Bodies in the Urine

The presence of ketone bodies in the urine is not strictly diagnostic for diabetes mellitus. Ketone bodies are always excreted in small amounts, and particularly during fasting. As a rule, there is no glucose in the urine during fasting, so that the presence of glucose together with ketone bodies is quite specific for diabetes mellitus. Semi-quantitative estimations of ketone bodies in the urine are of great importance for the initial stabilization of diabetes mellitus.

Rothera's or Galat Test. Urine is added to ammonium sulfate and sodium nitroprusside, both in the form of dry powder. Ammonia is then added. A purple-red color, the intensity of which increases with rising concentrations, is formed in the presence of ketone bodies. A concentration as low as 3 mg% gives a weak positive reaction. *Acetest tablets* make use of the same principle. They also contain sodium nitroprusside. A drop of urine is put on a tablet. A purple color forms when more than 10 mg% acetoacetic acid or 25 mg% acetone are present in the urine. The paper strips, *Ketostix,* make use of the same principle. The strips are quickly dipped in the urine and the reaction read off within 15 sec. A weak purple-red color is formed in the presence of a concentration of 10 mg% acetoacetic acid.

3. Methods of Estimating Blood Sugar, and their Use in Diagnosing Diabetes Mellitus

Precautions mentioned in the introduction must be observed in interpreting the blood sugar and glucose tolerance. Apart from this, correct collection and storage of blood are also of considerable importance and the method used for estimation of glucose must be specific. The glucose concentration in blood left to stand at room temperature rapidly diminishes and is approximately halved within 3–4 hours. Addition of sodium fluoride prevents glycolysis in the red cells. 20 mg of a mixture of 1 part of sodium fluoride and 2 parts of potassium oxalate are used for 5 ml of blood and not only prevent coagulation of blood, but also guarantee that the glucose concentration remains constant. Immediate deproteinization of the blood is even better. Today, only those methods of estimating blood sugar which guarantee a high degree of specificity through the quality of deproteinization or through the specific measurement of glucose itself should be in use. HAGEDORN-JENSEN'S, HOFFMANN'S and the orthotolidine methods have been adapted to the autoanalyzer and are, therefore, most commonly used. Glucose oxidase methods are very good and are employed in nearly all laboratories. The hexokinase method which is absolutely specific and most reliable, is expensive and, therefore, used only for particular research purposes.

a) Fasting Blood Sugar

The fasting blood sugar is elevated in manifest diabetes mellitus. In the healthy control subject, the fasting blood sugar lies between 60 and 105 mg%. Values between 105 and 130 mg% are suggestive, but not yet diagnostic, of diabetes mellitus. Values over 130 mg% are usually diagnostic. In spite of this, diabetes mellitus must not be diagnosed on the basis of a single laboratory observation, since there are many sources of laboratory errors. Moreover, it is always possible that the patient was not fasting. Therefore, the diagnosis of diabetes mellitus is not based on a single blood-sugar value, but on several estimations of fasting blood sugar or on a glucose-tolerance curve in borderline cases.

b) Tests which Indirectly Determine the Dynamics of Insulin Secretion

α) Oral Glucose Tolerance Test (Fig. 19)

This test is usually carried out with 100 g of glucose per os in U.S.A., and with 50 g or 1.75 g/kg ideal body weight on the continent. The large dose of 100 g carries the disadvantage of occasional nausea and vomiting or diarrhea.

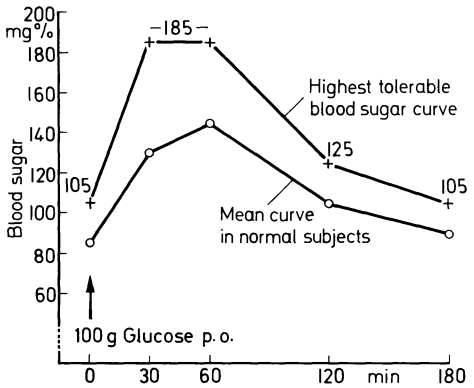


Fig. 19. Oral glucose tolerance test with 50 or 100 g and capillary (arterial) blood sugar determination

In children, 1.75 g of glucose/kg are usually given. Glucose is dissolved in 250 ml (50 g) or 500 ml (100 g) of water and is administered to the patient after a period of 12 hours of fasting. Blood sugar is estimated in the fasting state, 1/2, 1 and 2 hours after glucose intake. The person under investigation sits or lies still, drinks and eats nothing during the test and does not smoke. Diabetes is probable if the value after 30 min or after 1 hour is above 185 mg% and if the value after 2 hours is above 125 mg% (capillary blood). As was mentioned in the introduction, the age of the patient must be taken into consideration in the interpretation of these values. The values quoted are absolutely normal for a person between 60 and 80 years, whereas they are suggestive of prediabetes in someone between 20 and 30.

β) Intravenous Glucose Tolerance Test (Fig. 20)

The intravenous glucose tolerance test is a simple rapid test. In addition, it has the advantage over the oral glucose tolerance test that one

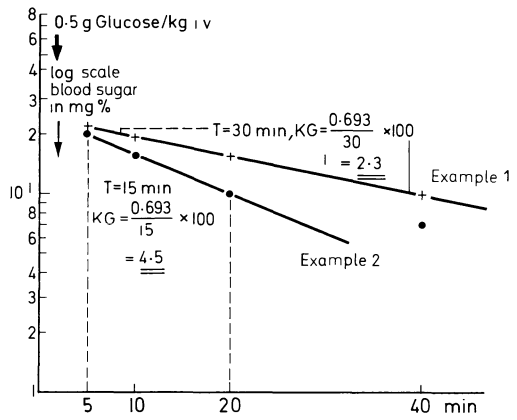


Fig. 20. Intravenous glucose tolerance test according to CONARD with two practical examples and the calculation of the glucose assimilation coefficient (KG)

factor of uncertainity, i.e. glucose absorption, is excluded. However, since it is known that glucagon, secretin and other intestinal factors also affect insulin secretion, it has become questionable whether the intravenous glucose tolerance test really has so many advantages over the oral test. However, it appears that the results of the two tests agree very well. As with the oral tolerance test, a normal diet with at least 200 g of carbohydrates during the 3 days prior to the test is required. The test is carried out in the morning in the fasting state. Capillary blood is collected at the start and then 0.5 g glucose per kg of body weight in a 40 or 50% solution is rapidly injected intravenously (approximately 2 min). Further blood collections are made after 10, 20 and 40 min. The blood-sugar levels are then plotted on semi-logarithmic paper. The glucose-assimilation coefficient (KG) may be calculated with the aid of logarithm tables. It is simpler to use semi-logarithmic paper, on which the blood sugar is plotted on the ordinate and the time in minutes on the abscissa. The line obtained from the three blood-sugar values may be extended and the biological half-life (T) is read from the curve. For this, one chooses 2 fixed points on the line, e.g. the blood sugar value of 200 and 100 mg%, and from the abscissa (T) reads off the minutes required for this decrease in blood sugar. The glucose assimilation coefficient (KG) may then be calculated as follows:

$$KG = \frac{0.963}{T \text{ in min}} \times 100 .$$

Normal Values. Normal values are still rather poorly standardized for different age groups. Glucose assimilation decreases with increasing age. The mean KG value is 1.80. A KG value of less than 1.40 is very suggestive of diabetes (= average value of a large group − 2 standard deviations). Whereas we have to interpret 3 figures in the oral glucose tolerance test (blood sugar at 30, 60 and 120 min), we obtain a single value (KG) with the intravenous tolerance test, which may be interpreted without taking any other values into consideration.

γ) Tolbutamide Test

When given intravenously, sulfonylureas cause a rapid release of insulin and a fall in blood sugar. In the diabetic, less insulin is secreted and the fall of the blood sugar is less marked and slower. The blood sugar reaches its lowest value after 60 min or even later. A specimen for the fasting blood sugar is collected and then 1 g of tolbutamide is given intravenously over a period of 2–3 min. Blood-sugar estimations are made after 20, 30, 40 and 60 min. In healthy subjects, the blood sugar falls rapidly and reaches a minimum between 20 and 40 min. Unfortunately, this test is not quite as reliable as the glucose tolerance test. In general, it can be taken that after 20 min the blood sugar value should be less than 75% of the original value. If it is higher than 90%, diabetes can be diagnosed with certainty. Further tests to detect the presence of diabetes mellitus must be employed in patients with values between 75 and 90% of the fasting blood sugar value after 20 min. A value higher than 75% of the fasting value at 30 min is considered definitely abnormal.

δ) Cortisone Glucose Tolerance Test

Glucocorticoids are some of the diabetogenic factors which can convert prediabetes into latent or subclinical diabetes. The test is carried out as follows. The diet should contain at least 200 g of carbohydrate in the last three days before the test. The patient receives 50 mg of cortisone acetate $8\frac{1}{2}$ and 2 hours before the glucose tolerance test. 100 g of glucose are given orally for the glucose tolerance test. The test can be interpreted as being positive for diabetes when the value after 1 hour (venous blood) rises above 160 mg%, and the value after 2 hours exceeds 140 mg%. These values exceed the glucose values normally found in the venous

blood during the normal oral glucose tolerance test with 100 g of glucose by 20 mg%. The cortisone glucose-tolerance test has not become a routine test and remains a research instrument.

ε) Estimation of Insulin in the Plasma (Fig. 21)

Insulin was the first peptide hormone in the plasma to be measured by the radioimmunoassay, and BERSON and YALOW are to be credited with this success. The method is based on the isotope-dilution principle. A predetermined limited amount of insulin antibodies is mixed with serum containing insulin and a certain amount of insulin labeled with iodine 131. Insulin antibodies of the guinea pig bind insulin labeled with iodine 131. More labeled insulin is bound if there is little insulin in the serum. The antibodies cannot distinguish between insulin labeled with iodine 131 and human unlabeled insulin. At the end of the reaction, when an equilibrium is reached between labeled insulin, unlabeled insulin, antibodies and antibody-insulin complexes, the free insulin labeled with iodine 131 is separated from antibody-bound insulin. This separation is achieved either by means of paper electrophoresis or by a second antibody reaction in which guinea pig antibodies against insulin are precipitated by anti-guinea pig serum. A standard curve is made with human unlabeled insulin. The insulin concentrations in serum with unknown insulin contents are read off from the curve. The concentration of the so-called immunoreactive or immunological insulin in the serum lies between 10 and 20 μU/ml of plasma in the normal fasting human. The average concentration is about 15 μU/ml of plasma. During the intravenous glucose tolerance test, the insulin levels rise rapidly within 5 min and reach a maximum of 50–100 μU. They then fall to the original values after 20–40 min. After oral intake of glucose, the insulin values in the blood do not reach a maximum until after 30–60 min, and they fall much more slowly. After 120–150 min they are back to the fasting value. Insulin secretion does not react uniformly to glucose loads in diabetes. The fasting values are diminished in juvenile ketotic diabetes, and the insulin concentration does not rise after glucose administration. In the elderly diabetic where the disease is manifest but ketosis is absent, the fasting insulin values are often within the normal range. The rise in insulin concentration is delayed, but in obese subclinical diabetics it can occasionally reach values exceeding those found in normal controls after 2–3 hours. In young prediabetic persons who demonstrate a normal glucose tolerance but are genetically diabetics, the rise in insulin concentration during the glucose tolerance test is on an average less than in normal controls. The differences are, however, slight. They are of statistical value for groups of patients, but are of no diagnostic value for individuals. Insulin estimation in the plasma has, therefore, remained a research instrument rather than a diagnostic tool.

G. Hypoglycemia

E. R. FROESCH

1. Definition

The blood sugar varies from 70 to 130 mg% according to the nutritional state. By definition, one speaks of hypoglycemia when the blood sugar falls below 50 mg%. The true fasting glucose lies between 70 and 105 mg% when measured by a specific enzymatic method. The blood sugar may fall below 70 mg% during prolonged fasting, but usually not below 50 mg%. The symptoms of mild hypoglycemia are nonspecific and cannot be distinguished from

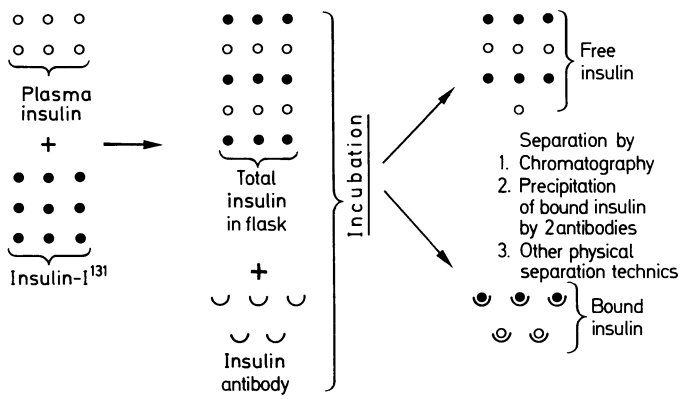


Fig. 21. Principle of the radioimmuno assay of insulin in serum

those of neurovegetative dystonia. It is therefore not permissible to diagnose hypoglycemia on the basis of symptoms such as sweating, trembling, palpitations, and giddiness alone. The diagnosis is based on at least one, or better several, blood sugar levels below 50 mg%.

2. Symptoms of Hypoglycemia

The symptoms of hypoglycemia are almost entirely cerebral in origin, since the brain is primarily dependent on glucose for its energy supply, whereas most other tissues turn to other sources of energy when glucose is scarce. Myocardial infarction occasionally described as having occurred during hypoglycemia is not due to nonavailability of glucose in itself, but rather to the endocrine counterregulation and particularly to increased levels of blood catecholamines. The sensitivity of different areas of the brain to hypoglycemia seems to vary. The hypothesis that the cerebral centers suffer from hypoglycemia in the reversed order of their phylogenetic development is interesting but should not be interpreted literally. The bizarre and varying symptoms of hypoglycemia make such a strict order of successive failure of one brain center after another during hypoglycemia seem unlikely. As a rule, however, the function of the cerebral cortex is always lost first, whereas the respiratory center, for example, may continue to function long after the complete loss of cortical function. The clinical picture of hypoglycemia depends on the rate at which the blood sugar falls. The signs of catecholamine release are often much more pronounced during rapidly developing hypoglycemia than during hypoglycemia which arises slowly. Symptoms due to adrenaline are cold sweating, trembling, uneasy feeling, nausea, occasionally hunger, palpitations and a striking pallor of the skin. The cerebral disturbance varies and is again dependent on the type and manner of the fall in blood sugar. Common neurologic symptoms are disorders of coordination leading to ataxia, sensory and motory disturbances and diplopia. Hemiparesis may be seen. The parts of the brain with a poor blood supply appear to fail first. Hypoglycemia often begins with apathy and inadequate reactions, clouding of consciousness and finally ends in deep coma with a characteristic retrograde amnesia. Not uncommonly, the patients are first irritated and excited, with outbursts of anger or with peculiar unintelligible behavior leading to the diagnosis of schizophrenia. Hypoglycemia may provoke any type of epileptic seizure and many patients with organic and other forms of hypoglycemia reach the internist in a roundabout way via the psychiatrist. In hypoglycemic shock of short duration no permanent brain damage remains. The prognosis becomes critical when the coma lasts for half an hour, one hour or longer. The patients then no longer awake promptly when the blood sugar level is normalized. A physician should never give up when the patient does not awake readily from the hypoglycemic shock or within an hour after correction of hypoglycemia. There are many examples of patients with prolonged coma whose cerebral function was restored to normal only after 24, 48 hours or even after weeks. In this respect, hypoglycemia may be compared with ischemia of the brain, in which several weeks may be needed for complete recovery. If a patient regains consciousness after an injection of glucose but becomes unconscious later on, the blood sugar has probably fallen to hypoglycemic values again due to the prolonged action of insulin. In most cases of hypoglycemia, the glucose injection should be followed by an intravenous drip of 5 or 10% glucose. The so-called protracted hypoglycemic shock is an extremely dangerous complication of hypoglycemia. The coma is described as protracted when consciousness is not regained within an hour after normalization of the blood sugar. The longer hypoglycemia lasts, the greater is the danger of irreversible cerebral damage. Patients with panhypopituitarism and cortical adrenal insufficiency are particularly liable to develop protracted hypoglycemic shock; since such patients often respond very well to treatment with glucocorticoids, prednisone has been included in the therapeutic scheme for protracted hypoglycemic shock. The prognosis is poor but not utterly hopeless. One observes again and again that patients who have already been given up wake up from hypoglycemic coma which has lasted for several days and that they recover completely or almost completely.

3. Treatment of Hypoglycemic Shock

Since the prognosis of hypoglycemic shock worsens within minutes, adequate amounts of glucose should be given at once without waiting for laboratory results. A single intravenous injection of 20–40 g of glucose is frequently sufficient to correct hypoglycemia. If the patient does not wake up at once, glucose is administered by slow intravenous infusion and the blood sugar is measured hourly. The treatment can be combined with administration of glucagon, 1–2 mg being given intravenously or intramuscularly. A blood sugar stabilized between 150 and 300 mg% is the only criterion indicating that therapy is adequate.

Therapeutic Scheme for Hypoglycemic Shock

1. 20–40 g of glucose (50–100 ml 40% glucose) intravenously.
2. Intravenous drip of 5–10% glucose so that blood sugar is kept constant between 150 and 300 mg%.
3. Crystalline glucagon, 1–2 mg every 2 hours, intramuscularly or by slow i.v. infusion
4. 30 mg of water soluble prednisone every 6 hours intramuscularly or in the drip.

Hypoglycemia in the presence of panhypopituitarism and adrenal insufficiency must be treated with physiological saline as well as glucose. An aqueous glucose solution leads to severe water intoxication and hyponatremia.

4. General Pathophysiology of Hypoglycemia

Hypoglycemia is due to an imbalance between exogenous glucose intake and endogenous glucose formation on one hand, and glucose utilization on the other hand. Diminished gluconeogenesis nearly always results in hypoglycemia, whereas increased glucose utilization is often compensated for a long time by increased hepatic glucose release. Hypoglycemia may be subdivided into fasting and reactive types, depending on whether it develops in the fasting state, after food intake or after the action of a certain agent (see Table 27).

5. Reactive Hypoglycemia

Reactive hypoglycemia hardly comes into the differential diagnosis of organic hyperinsulinism. Leucine stimulates not only normal B-cells, but also B-islet cell adenomas (FLOYD, 1964, 1966). This amino acid causes hypoglycemia more or less regularly in such patients. Patients with *familial leucine hypersensitivity* (COCHRANE, 1956) also react to leucine with excessive secretion of insulin (YALLOW, 1960) which leads to hypoglycemia in these children in the absence of histologically detectable changes of the islets of Langerhans. These children often show a slight tendency to fasting hypoglycemia (COCHRANE, 1956). The mode of action of leucine is unknown, as is the biochemical substrate for the dysfunction of islet cells in leucine-hypoglycemia. A qualitatively normal, but quantitatively excessive response of the islet cells to a physiologic stimulus seems to be involved.

There are two distinct types of *glucose-induced reactive hypoglycemia.* Reactive hypoglycemia in the non-diabetic is characterized clinically by a feeling of weakness, giddiness, trembling, hunger, nervousness, nausea, headache and other unspecific symptoms 1 to 2 hours after breakfast and less commonly after lunch and dinner. Symptoms last between 10 and 30 min and disappear spontaneously. Many of these patients are emotionally labile. Although the blood sugar seldom reaches values below 60 mg%, these patients often react promptly to glucose administration. Many respond well to a diet low in carbohydrate and rich in protein. Meals should be small but frequent. The pathogenesis of reactive hypoglycemia is not uniform and in most cases not understood. Since no measurable disorder of glucose metabolism is

Table 27. Classification of hypoglycemia by pathogenesis

Fasting hypoglycemia	
With hyperinsulinism	Without hyperinsulinism
a) Organic hyperinsulinism with B islet-cell adenoma and carcinoma	Inborn errors of metabolism with defects of glycogenolytic enzymes:
b) Functional hyperinsulinism in newborns of diabetic mothers	a) Glycogenosis type I (G-6-phosphatase deficiency) b) Glycogenosis type III (debranching enzyme deficiency) c) Glycogenosis type IV (hepatic phosphorylase deficiency)
	Inborn errors of metabolism with defects of gluconeogenetic enzymes
	a) Fructose-1,6-diphosphatase deficiency
	Deficiency of endocrine insulin antagonists:
	a) Infantile hypoglycemia (Zetterström, adrenalin?) b) Pituitary dwarfs with growth hormone or ACTH deficiency c) Panhypopituitarism
	Paraneoblastic hypoglycemia: a) Tumor hypoglycemia
Reactive hypoglycemia	*Reactive hypoglycemia*
With hyperinsulinism	Without hyperinsulinism
a) Neurovegetative dystonia (mild) b) Diabetes mellitus (mild) c) Leucine-induced hypoglycemia	a) Hereditary fructose intolerance b) Fructose-1,6-diphosphatase deficiency c) Galactosemia

detectable in most patients, it is assumed that nervous, neurovegetative and psychological factors play an important role. A therapeutic trial with frequent meals with low carbohydrate content and psychopharmacological drugs is indicated in every case. A critical and complete survey of the problem of reactive hypoglycemia is that by MARKS and ROSE (1965, pp. 136–154).

The symptoms of reactive hypoglycemia occuring 1–2 hours after meals in gastrectomized patients cannot usually, as was previously assumed, be explained by truly low blood-sugar levels or a particularly rapid fall in blood sugar (STALKER, 1959; SMITH, 1953). Personality changes seem to be of importance in these cases too.

The other form of reactive hypoglycemia is typical of diabetics. In our experience, reactive hypoglycemia in early stages of diabetes is uncommon. It occurs mainly in overweight diabetics in the stage of prediabetes or subclinical diabetes (SELTZER, 1956). The blood sugar rises slowly, remains elevated for a longer period than normal and induces a delayed and prolonged release of insulin (PERLEY, 1967; BAGDADE, 1967), which infrequently results in mild hypoglycemia 3 to 6 hours after glucose intake. This form of hypoglycemia is never dangerous. It is temporary and clears up spontaneously. Treatment consists of frequent, small meals with low carbohydrate content.

In *hereditary fructose intolerance,* fructose-1-phosphate aldolase is absent in the liver. Fructose-1-phosphate accumulates in the liver after fructose intake. This intermediate blocks glycogenolytic and gluconeogenetic enzymes, so that the liver loses its property to replace the glucose that is utilized by other tissues. Hypoglycemic episodes in patients with hereditary fructose intolerance are severe and last for hours when large amounts of fructose are absorbed. Plasma insulin is not increased and even decreases during hypoglycemia. Patients with hereditary fructose intolerance develop a very strong distaste for sweet foods since they regularly vomit after fructose intake. In this way they protect themselves from the ill effects of the noxious agent which kills infants who are forced to take fructose over prolonged periods of time (FROESCH, 1972).

Fructose-1,6-diphosphatase deficiency may present itself with hypoglycemia as the leading symptom. Often lactic acidosis is more prominent. These children maintain normal blood sugar during fasting for as long as glycogen is available. When gluconeogenesis should set in they rapidly develop severe hypoglycemia. Fructose also induces acute hypoglycemia, probably by fructose-1-phosphate inhibition of phosphorylase. Children with this disease grow normally despite of fructose intake for which they do not develop a distaste, in contrast to subjects with hereditary fructose intolerance.

In contrast to hereditary fructose intolerance, hypoglycemia after galactose intake by *galactosemic children* is mild and usually symptomless. In these patients, galactose-1-phosphate accumulates in liver cells and inhibits the hepatic release of glucose, although to a lesser extent than fructose-1-phosphate (ISSELBACHER, 1966).

Sulfonylureas stimulate insulin secretion. Even in reasonable doses they occasionally induce severe and sometimes fatal hypoglycemia. Individual disturbances in their breakdown in the liver, renal insufficiency and stimultaneous administration of other drugs may be responsible for an excessive response to sulfonylureas (MARKS and ROSE, 1965). *Biguanides,* whose mode of action is still unexplained, are much less potent and hypoglycemic incidents are unknown after these agents. Salicylic acid itself has a weak hypoglycemic effect, but can potentiate the action of insulin and sulfonylureas. *Hypoglycine* is consumed with the fruit Blighia sapida in Jamaica, which contains other psychotropic agents. Hypoglycine and its metabolites, shortchain, unsaturated fatty acids, with terminal vinyl groups, react with coenzyme A. These peculiar fatty acids are not oxidized but block the oxidation of normal, endogenous fatty acids. Thus, glucose becomes the main source of energy and glucose utilization increases. In addition, gluconeogenesis is inhibited so that hypoglycemia develops (JELIFFE, 1954; CORREDOR, 1967). The reaction to *alcohol* differs according to the nutritional state of the individual. Large amounts of alcohol inhibit gluconeogenesis in fasting subjects and can cause severe hypoglycemia (FREINKEL, 1965; ARKY, 1966).

6. Fasting Hypoglycemia

Fasting hypoglycemia denotes clinical conditions in which a fall in blood sugar is in no way connected with the intake of food or drugs. Hypoglycemia develops during fasting or during work, when glucose utilization is increased and no glucose is absorbed from the gut. In normal subjects, the blood sugar does not fall below 70 mg% during the night. It may fall further, to 50–60 mg%, if fasting is continued for several days. Values below 50 mg% are not found in metabolically healthy people and are suggestive of an endocrine or metabolic disorder. Complete starvation leading to severe cachexia in the terminal stage may result in serious impairment of gluconeogenesis and hypoglycemia, even in subjects with previously sound metabolism.

Intense physical exercise can lead to a temporary fall in blood sugar which is rapidly compensated and, at the most, results in subjective symptoms of hypoglycemia, such as a feeling of emptiness and weakness.

Decreased levels of endocrine antagonists to insulin frequently lead to hypoglycemia. Hypoglycemia is most severe in panhypopituitarism and may often be the cause of death. In patients lacking both growth hormone and adrenal cortical hormones, prolonged fasting regularly results in hypoglycemia. Hypoglycemia occurs less frequently and is less severe in Addison's disease, where pituitary function is intact.

It is interesting that hypoglycemia never develops in adrenalectomized patients who have lost the adrenal medulla but are substituted with cortisone. In these patients, small amounts of adrenaline are still formed by the sympathetic ganglia. The adrenaline response to hypoglycemia is decreased and delayed in the patients (VAN EULER, 1961).

In contrast to the classical opinion, according to which adrenaline is the main endocrine regulator of glycogenolysis, it is now thought that physiological doses of adrenaline may be ineffective and that glycogenolysis is mainly controlled by glucagon (SOKAL, 1966). Nevertheless, adrenaline is one of the major physiologic stimuli of lipolysis in adipose tissue and may hence promote gluconeogenesis through increased levels of free fatty acids. Free fatty acids are converted into acetyl coenzyme A in the liver. The ratio of acetyl coenzyme A to coenzyme A changes in favor of the former. Pyruvate now is mainly converted to oxalacetate instead of acetyl coenzyme A and oxalacetate is available for the formation of new glucose. BROBERGER and ZETTERSTRÖM (1961) assume that failure to release adrenaline may be the cause of some cases of spontaneous hypoglycemia in children. No single hormone deficiency can be held responsible for all cases of "*idiopathic infantile hypoglycemia*" (MCQUARRIE, 1954). Symptoms usually begin before the 6th month and disappear at the latest during puberty. Ephedrine, small doses of glucocorticoids and diazoxide can be therapeutically helpful in these patients. Diazoxide is certainly the most effective of these drugs, but has also some side effects (MARKS and ROSE, 1965). Infantile hypoglycemia in the congenital *adrenogenital syndrome* with 11-β-Hydroxylase deficiency is rare.

Hypoglycemia is a regular concomitant symptom of glycogenosis type I and is occasionally found in types III and VI. *Type-I glycogenosis* is inherited as an autosomal recessive trait. There is no glucose-6-phosphatase in the liver. This is the crucial enzyme for glucose production by the liver, since it is responsible for the hydrolysis of glucose-6-phosphate. These patients' mental development is more or less normal, provided that frequent small meals are given to prevent severe hypoglycemia (FIELD, 1966). The differential diagnosis between organic hyperinsulinism and the above-mentioned diseases presents no difficulties. Only leucine-hypersensitivity and idiopathic infantile hypoglycemia could be mistaken for organic hyperinsulinism.

Large extra-pancreatic tumors are rarely associated with hypoglycemia and they present greater problems in the differential diagnosis. The differential diagnosis between organic hyperinsulinism due to a B-cell adenoma and hypoglycemia due to an extrapancreatic tumor cannot be made on the basis of measurements of immunoreactive plasma insulin alone, since normal insulin values may sometimes be encountered in rare patients with islet-cell adenoma. Often, however, these extrapancreatic tumors are so large that they can hardly be overlooked. Two-thirds of the tumors are of mesenchymal origin, mainly fibrosarcomas, and lie within the retroperitoneal space or in the mediastinum (PAPAIOANNOU, 1966). Liver cell carcinomas come next in frequency of tumors causing hypoglycemia. They often lead to fulminating and severe hypoglycemia which may lead to death within a short time. The prognosis of hepatomas with hypoglycemia is, therefore, very bad. In contrast to this, mesenchymal tumors, although usually malignant, often grow rather slowly. Long remissions and even total cures after surgical treatment and irradiation of such tumors have been described. Carcinomas of the gastrointestinal tract, bronchus and adrenals rarely go along with hypoglycemia.

The pathogenesis of tumor hypoglycemia has not been fully explained (UNGER, 1966; FROESCH, 1968). On one hand, glucose utilization by the tumors is increased, on the other hand, glucose release from the liver into the blood is partly or completely blocked despite otherwise normal liver function and normal glycogen content of the liver (FROESCH, 1963; JAKOB, 1967). Pharmacologic doses of glucagon result in rapid glycogenolysis and in a rise in blood sugar. However, glucose cannot be mobilized spontaneously in adequate amounts. Furthermore, lipolysis, i.e. the release of free fatty acids from adipose tissue is partially or totally blocked so that free fatty acids do not increase during hypoglycemia. The tissues, therefore, continue to use glucose as their only available source of energy. This can result in severe hypoglycemia (FROESCH, 1963; JAKOB, 1967). In the normal subject, only about 20% of the total

calories are covered by glucose during fasting, whereas glucose remains the chief substrate in these patients with tumor hypoglycemia even during very severe hypoglycemia. The contribution of glucose to total CO_2-production during hypoglycemia was found to be 40–60%, and therefore greatly increased in two patients with tumor hypoglycemia. The relation between the block of hepatic glucose release and lipolysis has not yet been clarified in patients with tumor hypoglycemia. SILVERSTEIN, WAKIM, and BAHN (1965) found increased urinary excretion of tryptophane in patients with tumor hypoglycemia. L-tryptophane causes inactivation of phosphoenol pyruvate carboxykinase in the liver and thus blocks gluconeogenesis (RAY, 1966). On the other hand, L-tryptophane is partly decomposed to nicotinic acid which inhibits lipolysis in adipose tissue. This might explain both metabolic abnormalities, the deficiency of hepatic glucose production as well as inhibition of lipolysis. This hypothesis, however, has not yet been confirmed experimentally.

Treatment of these patients entails regular glucose administration. Glucose requirements can rise to 600 or even 800 g per day, so that 20 to 30 g of glucose must be administered hourly. These patients must be wakened and fed regularly at night, otherwise they will fall in deep hypoglycemia and may die. Whenever possible, the tumor should be radically removed and postoperative irradiation be carried out, although these tumors are not particularly X-ray sensitive. An operation is worthwhile even when radical removal of the tumor is not possible, since reduction of the tumor mass can result in an improvement in the metabolic state. Hypoglycemic attacks may disappear completely or become less frequent and less severe. Long-term treatment with glucocorticoids or glucagon has not proved useful. Diazoxide appears to have no favorable influence on hypoglycemia in this condition.

7. Hyperinsulinism, B-Islet-Cell Adenoma

Hyperinsulinism is difficult to diagnose. Sometimes, hypoglycemic symptoms or shock may occur only once to three times a year, so that the patient does not consult the physician at all or the physician may not consider further investigations necessary. Hypoglycemia usually develops slowly without any signs of adrenaline secretion so that the patient is not warned and is taken by surprise by very severe hypoglycemic shocks. About half the patients with organic hyperinsulinism are first referred to the psychiatrist or neurologist before the internist is consulted. B islet-cell adenomas are still being misdiagnosed as psychosis or epilepsy. The following sequence of diagnostic measures is to be used in diagnosing hyperinsulinism as quickly as possible (MARKS, 1968):

a) Diagnosis of B Cell Adenoma

1. Glucose tolerance test with 50 or 100 g of glucose orally, with additional blood sugar estimation after 4 hours to detect reactive hypoglycemia in diabetics. This test must be carried out at the beginning of the investigations for practical reasons, since glucose tolerance may become abnormal after subsequent diagnostic measures. The glucose tolerance curve in these patients paradoxically shows a diabetic pattern in about 50% of the patients (MARKS, 1961). Fasting plasma-insulin values are elevated in about half the patients. Their rise following glucose administration may be normal, excessive or not uncommonly less than normal (SAMOLS, 1963; LUNDBAEK, 1966). Thus, the diagnostic value of the glucose tolerance test is questionable but it should still be placed at the beginning of the diagnostic measures.

2. Tolbutamide test using 1 g intravenously. This test should only be carried out when the fasting blood sugar lies above 50 mg%. In patients with B islet-cell adenoma, the blood sugar falls below 30 mg% within 30 to 60 min and, in contrast to the curve for the normal subject, does not return spontaneously or in due time to normal levels (FAJANS, 1961). In this test a cannula, or better still a slow infusion with physiologic saline should be set up so that glucose and glucagon can be administered intravenously at any time. Insulin values must be measured before and then 2, 5, 10, 30 and 60 min after tolbutamide administration. They usually rise above 200 μU/ml in cases of islet-cell adenoma and remain elevated for 30 min or longer.

3. 72-hours fasting test. When the tolbutamide test is negative and no hypoglycemic symptoms are elicited, a 72-hours fasting test may directly follow the tulbutamide test. In cases of islet-cell tumors, hypoglycemic attacks occur between the 12th and 36th hour after the last meal in more than two-thirds of all patients. However, this test must be carried through to 72 hours in order to absolutely exclude B-cell adenoma. An important safety measure is the close surveillance of the patients during the night. When hypoglycemic symptoms are experienced, the blood sugar should be determined, and the test should be discontinued when the blood sugar value falls below 40 mg%.

4. Glucagon test. Glucagon strongly stimulates insulin secretion, and MARKS and SAMOLS (1967) made use of this to develop a convenient test for the diagnosis of hyperinsulinism. Insulin secretion is stimulated to a greater extent by glucagon in patients with B islet-cell adenoma than in normal subjects (MARKS, 1968). These authors consider the glucagon test as one of the best and least dangerous for the diagnosis of B islet-cell adenoma.

As already explained, two-thirds of all patients with organic hyperinsulinism can be diagnosed by determination of immunoreactive plasma insulin. Since the insulin concentration in the blood varies considerably, at least 3 to 5 fasting insulin levels should be determined in order to exclude or confirm hyperinsulinism. Plasma-insulin values must be interpreted with caution. Insulin secretion is controlled primarily by the blood glucose. Therefore, the ratio of plasma insulin to blood glucose is more meaningful than the insulin values themselves. It is characteristic of B-cell adenomas that plasma insulin stays elevated between 20–40 μU/ml at blood-sugar levels below 40 mg% when they should be below 10 μU/ml as in the normal subjects. Since we have no possibility of producing low blood-sugar levels other than with insulin, it is difficult to establish this correlation for low blood-sugar levels in metabolically healthy subjects.

The diagnosis of a B-cell adenoma in a diabetic, an extremely rare coincidence, presents special difficulties. In 1960, MOSS and RHOADES collected 23 such cases from the literature. The diagnosis was made only post mortem in 16 of these 23 cases. Insulin requirements generally diminish. In addition to severe hypoglycemia, reactive hyperglycemia with ketosis may also develop. Increased plasma-insulin concentration is of no diagnostic value, since such patients already have circulating insulin antibodies due to previous treatment with insulin. Theoretically, the fasting test should gradually lead to hypoglycemia in the diabetic with a B-cell adenoma, making the diagnosis possible.

b) Incidence, Frequency and Localization of B-Islet-Cell Adenomas (MARKS and ROSE, 1965)

The incidence of B islet-cell adenomas is in no way sex-linked. They are most often found in the age group between 20 and 50, but children may also be affected. In one case, the adenoma was claimed to be present from birth on. Several cases of B-cell adenomas in one family are not rare. These patients usually belong to the group of endocrine adenomatosis. Most adenomas have a diameter between 1 and 2 cm. Smaller adenomas may also cause hypoglycemia. B-cell adenomas are found in all portions of the pancreas with no predelection. Those on the surface of the pancreas can be distinguished from lymph nodes by their brown, blue or blueish-black colour.

5% of all B-cell adenomas are located outside the pancreas. They occur with decreasing frequency in the duodenum, stomach, jejunum, Meckel's diverticulum, ileum, and extremely rarely in the gallbladder, excretory biliary ducts, mesentery, omentum, transverse colon and other structures in the neighbourhood of the pancreas. Multiple, benign adenomas are rare. They often are not much larger than normal islets, but may occasionally reach a diameter between 1 and 3 cm. These adenomas are not hyperplastic islets (previously termed polynesia) but are true multiple neoplasms. Most of these cases belong to the syndrome of endocrine adenomatosis.

10–20% of all insulin-secreting pancreatic tumors are malignant. There are no definite histologic criteria for malignancy. Metastases confirm malignancy. The clinical course is very variable. Hypoglycemia is often the first symptom of an islet carcinoma. Sometimes the rapidly increasing severity and frequency of hypoglycemic attacks suggests a carcinoma rather than an adenoma. The illness can also progress slowly and may then not be differentiated from an islet-cell adenoma. Occasionally, anorexia and tumor cachexia precede hypoglycemia. B-islet cell carcinomas produce insulin, proinsulin and proinsulin-like material.

c) Pathophysiology of Hypoglycemia in Active B-Islet-Cell Adenoma

Hyperinsulinism produces hypoglycemia during fasting, due to increased glucose assimilation and an inhibition of hepatic glucose production. As explained above, glucose tolerance and glucose assimilation coefficient may be in the diabetic range (MARKS, 1961). What are the mechanisms leading to hypoglycemia in these patients? Obviously, there is an imbalance between the formation of new glucose in the liver and glucose consumption in peripheral tissues. In patients with B islet-cell adenomas presenting a diabetic glucose tolerance curve, insulin secretion is usually not stimulated by glucose. These adenomas are often unresponsive to glucose. The decreased glucose assimilation coefficient is in part due to a failure of the pancreas to increase insulin secretion after glucose administration. Insulin secretion is relatively constant, and higher than in normal fasting subjects. Hypoglycemia occurs during prolonged

fasting because the plasma insulin level remains slightly elevated and inhibits glucose release from the liver. Not uncommonly, the imbalance between glucose production and glucose consumption comes to a new equilibrium at a blood sugar of 40 mg% which may last for several hours before the blood sugar decreases further and hypoglycemic shock develops.

A relative insulin resistance of the peripheral tissues in patients with hyperinsulinism has already been discussed. Diabetic glucose assimilation coefficients may be found in patients with organic hyperinsulinism at a time when plasma-insulin values are greatly elevated. We still cannot say with certainty whether humoral insulin antagonists are responsible or whether a true tissue resistance to insulin is present.

d) Treatment of the B-Islet-Cell Adenoma

When a case of organic hyperinsulinism has been diagnosed, an attempt should be made to localize the islet-cell adenoma. The tumor can be visualized by a celiacography in the pancreas in 10–20% of all cases. As a rule, adenomas with a diameter of 1–2 cm are detected by this procedure. Smaller adenomas may be missed. Scintigraphic methods for localizing insulinomas have so far been unsuccessful. The correct diagnosis of B islet-cell adenoma is absolutely imperative for the surgeon. The surgeon must find the islet-cell adenoma by means of careful dissection of the whole pancreas. Unfortunately, about 5% of islet-cell adenomas are ectopic and lie outside the pancreas, so that they are usually missed by the surgeon. Previously, when exploration of the pancreas was negative, four-fifths of the pancreas was removed. This procedure can no longer be recommended. If the adenoma cannot be localized, no portion of the pancreas should be removed. The adenoma may well be found during a second exploration one or two years later, when the tumor has grown larger (STEINKE, 1968). Besides, a four-fifth resection of the pancreas involves a high risk. An operation according to Whippel, with total pancreatectomy and partial resection of the small intestine should not be carried out since such patients become invalids afterwards. β-cytotoxic substances may make a cure possible for these patients in the near future (Streptozotocine) (BRODER, 1973).

e) Symptomatic Treatment of Organic Hyperinsulinism

At present, there is no ideal symptomatic treatment for organic hyperinsulinism. Surgery stands unchallenged at the top of the list of possible therapeutic measures. Only when it fails, i.e. when the islet-cell adenoma is not found or is inoperable, should pharmacotherapy be tried. The drug of choice is diazoxide (FAJANS, 1966). A daily dose of 150–300 mg increases the blood sugar by an average of 10 mg%. Symptoms of hypoglycemia may be delayed for hours so that the majority of patients may be symptom-free. Unpleasant side effects of diazoxide are sodium retention, palpitations, and high blood pressure. These symptoms are usually prevented by rather large doses of a hyperglycemic saliuretic drug. There are, however, other unavoidable side effects of diazoxide, of which growth of lanugo-type hair on the face is one of the most distressing. It always develops in children and female patients and is found to be extremely unpleasant. Gastrointestinal symptoms can, but need not arise and usually do not necessitate discontinuation of diazoxide therapy. The appetite decreases in most patients. Regular glucose administration is no longer necessary and overweight patients regularly lose weight in the first months of diazoxide therapy. Glucagon raises the blood sugar only temporarily and for this reason it is unsuitable for long-term treatment of organic hyperinsulinism. In contrast, treatment with glucocorticoids has proved successful since glucose formation in the liver is stimulated over a long period of time and blood sugar is increased. The risks of prolonged administration of glucocorticoids in high doses are wellknown. Streptozotocine should be restricted to cases of B-cell carcinoma. Some rather striking improvements of hypoglycemia and tumor growth have been reported after Streptozotocine therapy.

H. Glucagon: The Second Pancreatic Hormone

E. R. FROESCH

MCLEOD (1922) was the first to notice that intravenous administration of certain insulin preparations caused an initial rise in the blood sugar. MURLIN and his team (1923) separated a substance with a hyperglycemic action from insulin preparations and named it "glucagon", since it mobilized glucose from the liver.

Glucagon was later concentrated from insulin preparations and pancreas extracts. STAUB, SINN, and BEHRENS succeeded in crystallizing glucagon in 1953, and in 1956 BROMER succeeded in determining its structure. After ACTH and insulin, glucagon is the third polypeptide hormone which has been synthetically produced in the test tube.

1. Chemistry of Glucagon

The pancreas contains only about 5–10 mg of glucagon per kg wet weight, or about one twentieth to one tenth of its content of insulin (SAMOLS, 1966). Glucagon is highly insoluble at a pH of 4–9. These were probably the main reasons for the long delay in the purification of the hormone. Glucagon which has been recrystallized repeatedly still contains small traces of insulin and vice versa. These small traces of insulin cannot be removed from glucagon preparations by ordinary methods of protein chemistry.

Glucagon consists of a chain of 29 amino acids without disulfide bonds. The isoelectric point lies between pH 7.5 and 8. Glucagon is soluble at pH 3–4 and is very stable. Insulin contaminants can be removed by incubation with alkali or with reducing chemicals containing SH-groups, such as glutathione or mercaptoethanol.

2. Physiology of Glucagon

a) Site of Origin

Glucagon is formed in the A-cells of the islets of the pancreas. Ligation of the pancreatic duct and atrophy of the exocrine tissue do not result in the loss of A-cells. Glucagon can still be extracted from the pancreas after the B-islet cells have been destroyed with alloxan (PINCUS, 1950). In the dog, there are no A-cells in the uncinate process, and glucagon is also absent in this area of the pancreas (BENCOSME, 1956). A substance similar to glucagon can also be obtained from the gastrointestinal tract. Intestinal glucagon is immunochemically and biologically related to, but not identical to pancreatic glucagon (UNGER, 1968). It is assumed that the argyrophilic cells in the gut, which are similar in appearance to the A-cells of the pancreas, secrete intestinal glucagon. Intestinal glucagon appears to have a greater molecular weight than pancreatic glucagon (UNGER, 1968). It reacts with antibodies of most animals immunized with pancreatic glucagon. However, there are already antibodies which can distinguish the two types of glucagon and which react with only one or the other.

b) Metabolism

The existence of two or more different substances in the serum with glucagon-like activity sheds some doubt on previous findings which were collected and interpreted under the assumption that there is only one pancreatic glucagon. The glucagon concentration in the peripheral blood lies between 0.5 and 6 ng/ml serum when measured by the most recent immunochemical methods (WEINGES, 1968). Glucagon in a concentration of as low as 1.0 ng/ml has a glycogenolytic action on the liver (SOKAL, 1966). Thus, concentrations of this hormone found in the blood are also effective on the liver.

Glucagon action on the perfused liver *in vitro* is of short duration. It appears to be rapidly degraded and inactivated by the liver. It seems to have a short half-life which has not been exactly determined. Blood sugar rises for 20–30 min after an intravenous injection of glucagon and then falls again. The duration of action is quite similar to that of insulin.

Glucagon has three main sites of action in the organism. The most important is the stimulation of glycogenolysis in the liver. Glucagon converts the inactive phosphorylase-b into active phosphorylase-a, so that glycogen is rapidly broken down and released as glucose from the liver into the blood (SUTHERLAND, 1951, 1965). It appears that pancreatic glucagon is the true physiological antagonist of insulin (FOA, 1968). When the blood sugar falls, pancreatic glucagon rises in the pancreatic veins and in the peripheral blood and causes the blood sugar to return to normal values (UNGER, 1968). According to SOKAL (1966), glucagon is much more effective than adrenaline and noradrenaline, which also have a glycogenolytic action and work through the same mechanism as glucagon.

According to the interesting observations of SAMOLS and others in the last few years, glucagon is not only an antagonist of insulin, but also in some way a synergist. When glucagon is given by rapid intravenous injection, insulin concentration in the blood rises rapidly and before the blood sugar increases (SAMOLS, 1965). Furthermore, again according to these authors, there is first a rise in the glucagon concentration in the blood and then a release of insulin following the administration of glucose (SAMOLS, 1965). It would now appear that glucagon is no longer an antagonist to insulin, but rather a stimulator of insulin secretion. This view is supported by the observation that glucagon also stimulates insulin secretion *in vitro* (VECCHIO, 1966). Since it has become possible to differentiate between pancreatic and enteral glucagon, evidence accumulates that the rise in glucagon in the blood following administration of glucose is due to an increase in the secretion of enteral glucagon rather than pancreatic glucagon (UNGER, 1968). This is also supported by the observation that glucagon rises only after oral administration of glucose and not after intravenous administration. It is plausible that liberation of enteral

glucagon is responsible for the considerably greater release of insulin after intake of glucose by the oral route than after the intravenous administration of glucose. It is not known whether this enteral glucagon released after glucose administration has a physiological action different from that of pancreatic glucagon. It seems that the glycogenolytic action of enteral glucagon is less potent than that of pancreatic glucagon (UNGER, 1968). The third action of glucagon in the organism involves adipose tissue. Glucagon is a lipolytic hormone. In physiologic concentrations it stimulates the liberation of free fatty acids from adipose tissue. Here as well, glucagon appears to be an antagonist to insulin, which has a definite antilipolytic action. Insulin promotes the storage of absorbed food in the form of glycogen and fat, whereas glucagon stimulates the mobilization of these substances as glucose and free fatty acids (WEINGES, 1968; LEFEBRE, 1967).

The mechanism of action of glucagon on adipose tissue, liver and islet cells appears to be the same. The mode of action of catecholamines and of glucagon has been thoroughly investigated, in particular by SUTHERLAND and his team (1965). According to these investigators, glucagon promotes the formation of cyclic 3′,5′-AMP from ATP through the activation of adenylcyclase. The cyclic AMP then converts inactive phosphorylase-b or inactive lipase into the active form of the enzyme by phosphorylation of serine residues in the enzyme. Protein kinases are responsible for this phosphorylation.

3. Overproduction of Glucagon

Overproduction of glucagon is sometimes held responsible for the development of diabetes mellitus (WEINGES, 1968). The prevalence of A-cells over B-cells in the pancreas of the diabetic was quoted in support of this hypothesis (FERNER, 1953). However, permanent diabetes mellitus has been successfully produced in experimental animals only in a few instances and with extremely high doses of glucagon. Glucagon levels in the blood of diabetics are usually slightly elevated or within normal limits. This is further evidence against the pathogenetic significance of glucagon in diabetes mellitus (WEINGES, 1968).

Although it is highly probable that overproduction of glucagon has nothing to do with essential diabetes mellitus, there have been a few reports of pancreatic tumors associated with hyperglycemia and glycosuria (UNGER, 1964; MCGAVRAN, 1966; YOSHINAGA, 1966). Usually these tumors are diffusely growing neoplasms of the A-cells which lead to destruction of the pancreas and to metastases in the liver. The estimation of glucagon content of the serum, and when possible of the tumor, is the only safe means of substantiating that these tumors really secrete glucagon and are responsible for the diabetic state. In the few cases described, the blood sugar showed very large swings and there was no ketosis. In addition, the cases were characterized by failure to respond to intravenous administration of glucagon.

4. The Glucagon-Deficiency Syndrome

Pancreatectomy results in diabetes and not in hypoglycemia. There is no satisfactory method, neither surgical nor chemical, to selectively eliminate the A-cells. Cobalt chloride, phenolsulfonphthalein and syntaline A are only partially effective. In certain types of birds, islets containing A-cells are separated from those containing B-cells. In these animals, fatal hypoglycemia can occasionally be produced by complete removal of the islets containing A-cells. It is assumed that this type of hypoglycemia is due to glucagon deficiency (FOA, 1968). There is still no good evidence that certain forms of spontaneous hypoglycemia during infancy are due to a glucagon deficiency.

References

Embryology and Histology

BARGMANN, W.: Die Langerhansschen Inseln des Pankreas. In: Handbuch der mikroskopischen Anatomie des Menschen, Bd. VI/2. Berlin: Springer 1939.

BENSLEY, R. R.: Studies on the pancreas of the guinea pig. Amer. J. Anat. **12**, 297 (1911/12).

CARAMIA, F.: Electron microscopic description of a third cell type in the islet of the rat pancreas. Amer. J. Anat. **112**, 53—64 (1963).

CREUTZFELDT, W., SCHMIDT, W.: Über die Wirkung von Kobaltchlorid auf den Blutzucker und die Pankreasinseln bei verschiedenen Nagetieren. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **222**, 487 (1954).

FERNER, H.: Das Inselsystem des Pankreas. Stuttgart: Georg Thieme 1952.

HARD, W.: The origin of differenciation of the alpha and beta cells in the pancreatic islets of the rat. Amer. J. Anat. **75**, 369 (1955).

KÖNIGSBERG, J. R.: The effects of early pituitary removal by "decapitation" on carbohydrate metabolism in the chick embryo. J. exp. Zool. **125**, 151—169 (1954).

LACY, P. E.: Electron microscopy of the beta cell of the pancreas. Amer. J. Med. **31**, 851 (1961).

LAZAROW, A.: Cell types of the islets of Langerhans and the hormons they produce. Diabetes **6**, 222 (1957).

MUNGER, B. L.: A phase and electron microscopic study of cellular differentiation in the pancreatic islets of the mouse. Amer. J. Anat. **103**, 275 (1958).

NEUBERT, K.: Bau und Entwicklung des menschlichen Pankreas. Wilhelm Roux' Arch. Entwickl.-Mech. Org. **111**, 29 (1927).

PETERSSON, B.: The two types of alpha cells during the development of the guinea-pig pancreas. Z. Zellforsch. **75**, 371—380 (1966).

WATARI, N.: Fine structure of nervous elements in the pancreas of some vertebrates. Z. Zellforsch. **85**, 291—314 (1968).
WILLIER, B. H.: Ontogeny of endocrine correlation. In: Analysis of development, hrsg. von B. H. WILLIER, P. A. WEISS und V. HAMBURGER. Philadelphia-London: W. B. Saunders Co. 1955.

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Reviews and Books

BOND, V. P.: Symposium on insulin. Amer. J. Med. **40**, 651—772 (1966).
CAMERON, M. P., O'CONNOR, M. (Hrsg.): The aetiology of diabetes mellitus and its complications. Ciba Foundation, Coll. on Endocrinology, vol. 15. London: J. & A. Churchill LTD 1964.
CHANCE, B., ESTABROOK, R. W., WILLIAMSON, J. R. (Hrsg.): Control of energy metabolism. New York-London: Academic Press 1965.
CRANE, R. K.: Intestinal absorption of sugars. Physiol. Rev. **40**, 789 (1960).
EXTON, J. H., PARK, C. R.: The stimulation of gluconeogenesis from lactate by epinephrine, glucagon and cyclic 3′, 5′-adenylate in the perfused rat liver. Pharmacol. Rev. **18**, 181 (1966).
FAJANS, S. S., FLOYD, J. C., JR., KNOPF, R. F., CONN, J. W.: Effect of amino acids and proteins on insulin secretion in man. Recent Progr. Hormone Res. **23**, 617 (1967).
FREHNER, H. U., FROESCH, E. R.: Diabetes-Fibel. Thieme, 1972.
FROESCH, E. R., BÜHLMANN, A., ROSSIER, P. H.: Das Coma diabeticum in heutiger Sicht. Verh. Deutsch. Ges. Inn. Med., 72. Kongreß. München: J. F. Bergmann 1966.
— BÜRGI, H., MÜLLER, W. A., HUMBEL, R. E., JAKOB, A., LABHART, A.: Nonsuppressible insulin-like activity in human serum: purification, physicochemical and biological properties and its relation to total serum ILA. Recent Progr. Hormone Res. **23**, 565 (1967).
GRÖNBERG, A., LARSSON, T., JUNG, J.: Diabetes in Sweden. A clinico-statistical, epidemiological and genetic study of hospital patients and death certificates. Acta med. scand., Suppl. **477** (1968).
JEANRENAUD, B., HEPP, D.: Adipose tissue. Regulation and metabolic functions. New York: Academic Press 1970.
KARLSON, P. (Hrsg.): Mechanism of hormone action. New York: Academic Press 1965.
KATSOYANNIS, P. G.: Synthetic insulins. In: PINCUS, G. (Hrsg.), IV. Insulin and pancreas, Recent Progr. Hormone Res. **23**, 505 (1967).
KLOSTERMEYER, H., HUMBEL, R. E.: Chemie und Biochemie des Insulins. Angew. Chem. **78**, 871 (1966).
LABHART, A.: Endogene und exogene Faktoren des Diabetes: Heredität, Adipositas, Zivilisation. Helv. med. Acta **32**, 349 (1965).
LANDAU, B.: Adrenal steroids in carbohydrate metabolism. Vitam. and Horm. **23**, 2 (1965).
LEIBEL, B. S., WRENSHALL, G. A. (Hrsg.): On the nature and treatment of diabetes. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.
LYNEN, F.: Mechanism and biological regulation of fatty acid synthesis. Progr. biochem. Pharmacol. **3**, 1 (1967).
LYNGSØ, J.: Serum insulin. A review. Acta med. scand. **179**, Suppl. **441**, 1 (1965).
PFEIFFER, E. F.: Handbook of Diabetes mellitus. Munich: J. F. Lehmann 1971.
RENOLD, A. E., CAHILL, G. F. (Hrsg.): Handbook of physiology, section 5, Adipose tissue. Washington D. C.: American Physiol. Society 1965.
— CAHILL, G. F., JR.: Diabetes mellitus. In: STANBURY, J. B., WYNGAARDEN, J. B., and FREDRICKSON, D. S. (Hrsg.), The metabolic basis of inherited disease, p. 69. New York: McGraw Hill Book Co. 1972.
— — GERRITSEN, G. C.: Second Brook Lodge Workshop on spontaneous diabetes in laboratory animals. Diabetologia **6**, 153–366 (1970).
— DULIN, W. E. (Hrsg.): Brook Lodge Workshop on spontaneous diabetes in laboratory animals. Diabetologia **3**, 63—266 (1967).
RODRIGUEZ, R. R.: Diabetes. Amsterdam: Excerpta Medica 1971.
STAUFFACHER, W., RENOLD, A. E.: Pathophysiology of diabetes mellitus. Im Druck.
STEINER, D. F., FREINKEL, N.: Handbook of physiology, Sect. 7: Endocrinology, vol. 1 on endocrine pancreas. Baltimore: Williams Wilkins and American Physiological Society 1972.
WHELAN, W. J., CAMERON, M. P. (Hrsg.): Control of glycogen metabolism. London: J. & A. Churchill, LTD. 1964.
WIELAND, O.: Formation of ketone bodies and its regulation. In: LEVINE, R., and R. LUFT (Hrsg.), Advances in metabolic disorders, vol. 3. Im Druck.
WILLIAMS, R. H., ENSINCK, J. W.: Secretion, fates and actions of insulin and related products. The Banting Memorial Lecture 1966. Diabetes **15**, 623 (1966).
YOUNG, F. G., BROWN, W. A., WOLFF, F. W. (Hrsg.): The mechanism of action of insulin. Oxford: Blackwell Scientific Publications 1960.
ZAHN, H.: Struktur und Synthese von Insulin. Verh. d. Dtsch. Gesell. f. Innere Medizin 1967, S. 800. München: J. F. Bergmann 1967.

1. Energy Exchange and Intermediary Metabolism

BUEDING, E., BÜLBRING, E.: Some biochemical properties of intestinal smooth muscle in relation to the physiological actions of epinephrine. In: B. CHANCE, R. W. ESTABROOK and J. R. WILLIAMSON (eds.), Control of energy metabolism, p. 313. New York and London 1965.
CRANE, R. K.: Intestinal absorption of sugars. Physiol. Rev. **40**, 789 (1960).
CROFFORD, O. B., RENOLD, A. E.: Glucose uptake by incubated rat epididymal adipose tissue. Rate limiting steps and site of insulin action. J. biol. Chem. **240**, 14 (1965).
DANFORTH, W. H.: Activation of glycolytic pathway in muscle. In: B. CHANCE, R. W. ESTABROOK and J. R. WILLIAMSON (eds.), Control of energy metabolism, p. 287. New York and London 1965.
FROESCH, E. R., GINSBERG, J. L.: Fructose metabolism of adipose tissue. I. Comparison of fructose and glucose metabolism in epididymal adipose tissue of normal rats. J. biol. Chem. **237**, 11 (1962).
HAVEL, R. J., NAIMARK, A., BORCHGRVINK, C. F.: Turnover rate and oxidation of free fatty acids of blood plasma in man during excercise: studies during continuous infusion of palmitate-1-C^{14}. J. clin. Invest. **42**, 1054 (1963).
HELMREICH, E., DANFORTH, W. H., KARPATKIN, S., CORI, C. F.: The response of the glycolytic system of anaerobic frog sartorius muscle to electrical stimulation. In: B. CHANCE, R. W. ESTABROOK and J. R. WILLIAMSON (eds.), Control of energy metabolism, p. 299. New York and London 1965.
— KARPATKIN, S., CORI, C. F.: Regulation of glycolysis in skeletal muscle. In: W. J. WHELAN and M. P. CAMERON (eds.), Control of glycogen metabolism. Ciba Foundation Symposium, p. 211, London 1964.
ILLINGWORTH, B., BROWN, D. H., CORI, C. F.: The influence of phosphorylase on the structure of glycogen. In: W. J. WHELAN and M. P. CAMERON (eds.), Control of glycogen metabolism. Ciba Foundation Symposium, p. 107, London 1964.

INGLE, D.J., MORLEY, E.H., STETTEN, D., JR.: Metabolism of glucose in resting and working rats. Amer. J. Physiol. **182**, 263 (1955).

KREBS, E.G., GONSALEZ, C., POSNER, J.B., LOVE, D.S., BRATVOLD, G.E., FISCHER, E.H.: Interconversion reactions of muscle phosphorylase b and a. In: W.J. WHELAN and M.P. CAMERON (eds.), Control of glycogen metabolism. Ciba Foundation Symposium, p. 200, London 1964.

LARNER, J., ROSELL-PEREIZ, M., FRIEDMAN, D.L., CRAIG, J.W.: Insulin and the control of UDPG-α-Glucan Transglucosylase activity. In: W.J. WHELAN and M.P. CAMERON (eds.), Control of glycogen metabolism. Ciba Foundation Symposium, p. 273, London 1964.

LELOIR, L.F.: Rôle of uridine diphosphate glucose in the synthesis of glycogen. In: W.J. WHELAN and M.P. CAMERON (eds.), Control of glycogen metabolism. Ciba Foundation Symposium, p. 68, London 1964.

LYNEN, F.: Mechanism and biological regulation of fatty acid synthesis. Progr. biochem. Pharmacol. **3**, 1 (1967).

MORGAN, H.E., REGEN, D.M., HENDERSON, M.J., SAWYER, T.K., PARK, C.R.: Regulation of glucose uptake in muscle. VI. Effects of hypophysectomy, adrenalectomy, growth hormone, hydrocortisone and insulin on glucose transport and phosphorylation of the perfused rat heart. J. biol. Chem. **236**, 2162 (1961).

OPIE, L.H., EVANS, J.R., SHIPP, J.C.: Effect of fasting on glucose and palmitate metabolism of perfused rat heart. Amer. J. Physiol. **205**, 1203 (1963).

PAUL, P., ISSEKUTZ, B., JR.: Role of extramuscular energy sources in the metabolism of the exercising dog. J. appl. Physiol. **22**, 615 (1967).

RANDLE, P.J., NEWSHOLME, E.A., GARLAND, P.B.: Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and diabetes and starvation on uptake and metabolic fate of glucose in rat heart and diaphragm muscle. Biochem. J. **93**, 652 (1964).

SANDERS, C.A., LEVINSON, G.E., ABELMANN, W.H., FREINKEL, N.: Effect of exercise on the peripheral utilization of glucose in man. New Engl. J. Med. **271**, 220 (1964).

STANBURY, J., WYNGARDEN, J.B., FREDRICKSON, D.S.: The metabolic basis of inherited disease, 2nd ed. New York-Toronto-London: McGraw Hill Book Company 1966.

UYEDA, K., RACKER, E.: Coordinated stimulation of Hexokinase and phosphofructokinase by phosphate in a reconstituted system of glycolysis. In: B. CHANCE, R.W. ESTABROOK and J.R. WILLIAMSON (eds.), Control of energy metabolism, p. 127. New York and London 1965.

WILBRANDT, W., ROSENBERG, T.: The concept of carrier transport and its corollaries in pharmacology. Pharmacol. Rev. **13**, 2 (1961).

WILLIAMSON, J.R.: Glycolytic control mechanisms. I. Inhibition of glycolysis by acetate and pyruvate in the isolated, perfused rat heart. J. biol. Chem. **240**, 2308 (1965).

2. Glucose Metabolism in the Liver

EXTON, J.H., PARK, C.R.: The stimulation of gluconeogenesis from lactate by epinephrine, glucagon and cyclic 3′-5′-adenylate in the perfused rat liver. Pharmacol. Rev. **18**, 181 (1966).

LARDY, H.A., FOSTER, D.O., YOUNG, J.W., SHRAGO, E., RAY, P.D.: Hormonal control of enzymes participating in gluconeogenesis and lipogenesis. J. cell. comp. Physiol., Suppl. 1 to **66**, 39 (1965).

— PAETKAN, V., WALTER, P.: Paths of carbon in gluconeogenesis and lipogenesis: The role of mitochondria in supplying precursors of phosphoenol-pyruvate. Proc. nat. Acad. Sci. (Wash.) **53**, 1410 (1965).

SOKAL, J.E.: Glucagon — an essential hormone. Amer. J. Med. **41**, 331 (1966).

STRUCK, E., ASHMORE, J., WIELAND, O.: Stimulierung der Gluconeogenese durch langkettige Fettsäuren und Glucagon. Biochem. Z. **343**, 107 (1965).

SUTHERLAND, E.W., ØYE, I., BUTCHER, R.W.: The action of epinephrine and the role of the adenyl cyclase system in hormone action. Recent Progr. Hormone Res. **21**, 623 (1965).

3. Chemistry, Synthesis and Effects of Insulin

ABEL, J.J.: Cristaline insulin. Proc. nat. Acad. Sci. (Wash.) **12**, 132 (1926).

BANTING, F.G., BEST, C.H.: The internal secretion of the pancreas. J. Lab. clin. Med. **7**, 251 (1922).

BEIGELMAN, P.M., HOLLANDER, P.B.: Effect of insulin upon resting electrical potential of adipose tissue. Proc. Soc. exp. Biol. (N.Y.) **110**, 590 (1962).

BÜRGI, H., KOPETZ, K., SCHWARZ, K., FROESCH, E.R.: Fate of rat insulin in rat-liver perfusion studied by adipose-tissue assay. Lancet **1963II**, 314.

— MÜLLER, W.A., HUMBEL, R.E., LABHART, A., FROESCH, E.R.: Non-suppressible insulin-like activity of human serum. I. Physicochemical properties, extraction and partial purification. Biochim. biophys. Acta (Amst.) **121**, 349 (1966).

BUTCHER, R.W., SNEYD, J.G.T., PARK, C.R., SUTHERLAND, E.W., JR.: Effect of insulin on adenosine-3′5′-monophosphate in the rat epididymal fat pad. J. biol. Chem. **241**, 1652 (1966).

CAHILL, G.F., JR., ASHMORE, J., EARLE, A.S., ZOTTU, S.: Glucose penetration into the liver. Amer. J. Physiol. **192**, 491 (1958).

CAMPBELL, J., RASTOGI, K.S.: Effects of growth hormone on the rate of disappearance of insulin from blood in depancreatomized and Houssay dogs. Metabolism **16**, 562 (1967).

CARRUTHERS, B.M., WINEGRAD, A.I.: Effects of insulin on amino acid and ribonucleic acid metabolism in rat adipose tissue. Amer. J. Physiol. **202**, 605 (1962).

CERASI, E., LUFT, R.: Insulin response to glucose loading in acromegaly. Lancet **1964II**, 769.

CHAMBERLAIN, M.J., STIMMLER, L.: The renal handling of insulin. J. clin. Invest. **46**, 911 (1967).

COMBES, B., ADAMS, R.H., STRICKLAND, W., MADISON, L.L.: The physiological significance of the secretion of endogenous insulin into the portal circulation. IV. Hepatic uptake of glucose during glucose infusion in nondiabetic dogs. J. clin. Invest. **40**, 1706 (1961).

CONARD, V.: Mesure de l'assimilation du glucose: bases théoriques et applications cliniques. Acta gastro-ent. belg. **18**, 655 (1955).

CROFFORD, O.B., RENOLD, A.E.: Glucose uptake by incubated rat epididymal adipose tissue: characteristics of the glucose transport system and action of insulin. J. biol. Chem. **240**, 3237 (1965).

CROUGHS, R.J.M., MAINGAY, D., TOUBER, J.L., RUYTER, H.A. DE, AALST, R. VAN: The effect of insulin binding antibodies on insulin-I^{131} metabolism. Acta endocr. (Kbh.) **50**, 213 (1965).

DANIEL, P.M., HENDERSON, J.R.: Insulin in bile and other body fluids. Lancet **1967I**, 1256.

DE BODO, R.C., STEELE, R., ALTSZULER, N., DUNN, A., BISHOP, J.S.: Effects of insulin on hepatic glucose metabolism and glucose utilization by tissues. Diabetes **12**, 16 (1963).

DUPRÉ, R., ROJAS, L., WHITE, J.J., UNGER, R.H., BECK, J.C.: Effects of secretion on insulin and glucagon in portal and peripheral blood in man. Lancet **1966II**, 26.

FAJANS, S.S., FLOYD, J.C., KNOPF, R.F., RULL, J., GUNTSCHE, E.M., CONN, J.W.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. J. clin. Invest. **45**, 481 (1966).

FANKHAUSER, S.: Insulinantikörper und Ketosetendenz beim Diabetes mellitus. Helv. med. Acta **30**, 500 (1963).

Fiftieth Anniversary Insulin Symposium. Diabetes **21**, Suppl. 2, 385–714 (1972).

FRITZ, I.B.: Insulin action. New York: Academic Press 1972.

FROESCH, E.R., BÜRGI, H., BALLY, P., LABHART, A.: Insulin inhibition of spontaneous adipose tissue lipolysis and effects upon fructose and glucose metabolism. Molec. Pharmacol. **1**, 280 (1965).

— — MÜLLER, W.A., HUMBEL, R.E., JAKOB, A., LABHART, A.: Nonsuppressible insulin-like activity of human serum: purification, physicochemical and biological properties and its relation to total serum ILA. Recent Progr. Hormone Res. **23**, 565 (1967).

— — RAMSEIER, E.B., BALLY, P., LABHART, A.: Antibody-suppressible and nonsuppressible insulin-like activities in human serum and their physiologic significance. J. clin. Invest. **42**, 1816 (1963).

— MÜLLER, W.A., BÜRGI, H., WALDVOGEL, M., LABHART, A.: Non-suppressible insulin-like activity of human serum. II. Biological properties of plasma extracts with non-suppressible insulin-like activity. Biochim. biophys. Acta (Amst.) **121**, 360 (1966).

— WALDVOGEL, M., MEYER, U.A., JAKOB, A., LABHART, A.: Effects of 5-methylpyrazole-3-carboxylic acid in adipose tissue. I. Inhibition of lipolysis, effects on glucose-, fructose- and glycogen metabolism in vitro and comparison with insulin. Molec. Pharmacol. **3**, 429 (1967).

GRABER, A.L., PORTE, D., JR., WILLIAMS, R.H.: Clinical use of diazoxide and mechanism for its hyperglycemic effects. Diabetes **15**, 143 (1966).

GRODSKY, G.M., BENNET, L.L.: Effect of glucose pulse, glucagon and the cations Ca^{++}, Mg^{++} and K^{+} on insulin secretion in vitro. J. clin. Invest. **45**, 1018 (1966).

HIRATA, Y., FRYISAWA, M., SATO, H., ASANO, T., KATSUKI, S.: Effects of intravenous injection of Xylitol on blood sugar, blood pyruvic acid and plasma insulin levels in the dog. Z. ges. exp. Med. **145**, 111 (1968).

HOLLENBERG, C.H.: Adipose tissue lipase II. In: RENOLD, A.E., and CAHILL, G.F., JR. (eds.), Handbook of physiology, section 5, Adipose tissue, p. 301. Washington: American Physiological Society 1965.

HUMBEL, R.E.: Biosynthesis of the two chains of insulin. Proc. nat. Acad. Sci. (Wash.) **53**, 4 (1965).

Institute of Biochemistry, Academia Sinica, Institute of organic Chemistry, Academia Sinica and Department of Chemistry, Peking University: The total synthesis of crystalline insulin. Kexue Tongbao **17**, 241 (1966).

JAKOB, A., HAURI, CH., FROESCH, E.R.: Nonsuppressible insulin-like activity in human serum. III. Differentiation of two distinct molecules with nonsuppressible ILA. J. clin. Invest. **47**, 2678 (1968).

JUNGAS, R.L.: Role of cyclic 3′, 5′-AMP in the response of adipose tissue to insulin. Proc. nat. Acad. Sci. (Wash.) **56**, 757 (1966).

— BALL, E.G.: Studies on the metabolism of adipose tissue XII. The effects of insulin and epinephrine on free fatty acid and glycerol production in the presence and absence of glucose. Biochemistry **2**, 383 (1963).

KARAM, J.H., GRODSKY, G.M., FORSHAM, P.H.: Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. Diabetes **12**, 197 (1963).

KATSOYANNIS, P.G., FUKUDA, K., TOMETSKO, A., SUZUKI, K., TILAK, M.: Insulin peptides. X. The synthesis of the B chain of insulin and its combination with natural or synthetic A chain to generate insulin activity. J. Amer. chem. Soc. **86**, 930 (1964).

KETTERER, H., EISENTRAUT, A.M., UNGER, R.H.: Effect upon insulin secretion of physiologic doses of glucagon administered via the portal vein. Diabetes **16**, 283 (1967).

KIRKHAM, K.E., HUNTER, W.M.: Radioimmunoassay methods. London: Churchill 1971.

KRAHL, M.E.: Stimulation of peptide synthesis in adipose tissue by insulin without glucose. Amer. J. Physiol. **206**, 618 (1964).

KUZUYA, T., KANAZAWA, Y., KOSAKA, K.: Plasma insulin response to intravenously administered xylitol in dogs. Metabolism **15**, 1149 (1966).

LACY, P.E.: Studies by electron microscope of secretory processes of islet cells. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 1. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.

LEVINE, R., GOLDSTEIN, M.S.: On the mechanism of action of insulin. Recent Progr. Hormone Res. **11**, 343 (1955).

LOUBATIÈRES, A.: The hypoglycemic sulfonamides: history and development of the problem from 1942 to 1945. Ann. N.Y. Acad. Sci. **71**, 4 (1957).

MADISON, L.L.: The role of insulin in controlling carbohydrate metabolism in the liver. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 129. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.

MAHLER, R., STAFFORD, W.S., TARRANT, M.E., ASHMORE, J.: The effect of insulin on lipolysis. Diabetes **13**, 297 (1964).

MALAISSE, W.J., MALAISSE-LAGOE, F., MAYHEW, D.: A possible rolte of the adenylcyclase system in insulin secretion. J. clin. Invest. **46**, 1724 (1967).

MANCHESTER, K.L.: Insulin and protein metabolism in muscle. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 101. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.

MAYHEW, D.A., WRIGHT, P.H., ASHMORE, J.: Regulation of insulin secretion. Pharmacol. Rev. **21**, 183 (1969).

MEIENHOFER, J., SCHNABEL, E., BREMER, H., BRINKHOFF, O., ZABEL, R., SROKA, W., KLOSTERMEYER, H., BRANDENBURG, D., OKUDA, T., ZAHN, H.: Synthese der Insulinketten und ihre Kombination zu insulinaktiven Präparaten. Z. Naturforsch. **18**b, 1120 (1963).

MERRIFIELD, R.B.: New approaches to the chemical synthesis of peptides. In: G. PINCUS (ed.), Recent Progress in Hormone Research, Laurentian Hormone Conference, p. 451. New York and London 1967.

MILNER, R.D.G., HALES, C.N.: The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. Diabetologia **3**, 47 (1967).

MORELL, B., FROESCH, E.R.: Fibroblasts as an experimental tool in metabolic and hormone studies. I. Growth and glucose metabolism of fibroblasts in culture. II. Effects of insulin and nonsuppressible insulin-like activity (NSILA-S) on fibroblasts in culture. Europ. J. Clin. Invest. **3**, 112, 119 (1973).

MORGAN, H.E., HENDERSON, M.J., REGEN, D.M., PARK, C.R.: Regulation of glucose uptake in muscle. I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated perfused heart of normal rats. J. biol. Chem. **236**, 253 (1961).

MORGAN, H.E., REGEN, D.M., PARK, C.R.: Identification of a mobile carrier-mediated sugar transport system in muscle. J. biol. Chem. **239**, 369 (1964).

OELZ, O., JAKOB, A., FROESCH, E.R.: Nonsuppressible insulin-like activity (NSILA) of human serum. V. Hypoglycaemia and preferential metabolic stimulation of muscle by NSILA-S. Europ. J. clin. Invest. **1**, 48 (1970).

PARK, C.R., MORGAN, H.E., HENDERSON, M.J., REGEN, D.M., CADENAS, E., POST, R.L.: The regulation of glucose uptake in muscle as studied in the perfused rat heart. Recent Progr. Hormone Res. **17**, 493 (1961).

PIAZZA, E.U., GOODNER, C.J., FREINKEL, N.: A re-evaluation of in vitro methods for insulin bio-assay. Diabetes **8**, 459 (1959).

PORTE, D., JR.: Beta adrenergic stimulation of insulin release in man. Diabetes **16**, 150 (1967).

— GRABER, A., KUZUYA, T., WILLIAMS, R.H.: The effect of epinephrine on immunoreactive insulin levels in man. Clin. Invest. **45**, 228 (1966).
— PUPO, A.A.: Insulin responses to glucose: Evidence for a two pool system in man. J. clin. Invest. **48**, 2309 (1969).
RENOLD, A.E.: Insulin biosynthesis and secretion. A still unsettled topic. New Engl. J. Med. **282**, 173 (1970).
— HASTINGS, A.B., NESBETT, F.B., ASHMORE, J.: Studies on carbohydrate metabolism in rat liver slices; biochemical sequence of events after insulin administration. J. biol. Chem. **213**, 135 (1955).
SAMOLS, E.: Immunochemical aspects of insulin. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 247. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.
— MARRI, G., MARKS, V.: Promotion of insulin secretion by glucagon. Lancet **1965II**, 415.
— TYLER, J., MARRI, G., MARKS, V.: Stimulation of glucagon secretion by oral glucose. Lancet **1965II**, 1257.
SANGER, F., THOMPSON, E.O.P., KITAI, R.: The amide groups of insulin. Biochem. J. **59**, 509 (1955).
SCHARFF, R., WOOL, I.G.: Accumulation of amino acids in muscle of perfused rat heart. Effect of insulin in the presence of puromycin. Biochem. J. **97**, 272 (1965).
SCHWARTZ, T.B., FLANAGAN, G.C., STUPPY, G.W., TARUM, D.W.: The hypoglycemic effect of L-leucine in organic hyperinsulinism. J. Lab. clin. Med. **54**, 944 (1959).
SIMON, E., KRAICER, P.F.: The blockade of insulin secretion by mannoheptulose. Israel J. med. Sci. **2**, 785 (1966).
SLOBIN, L.I., CARPENTER, F.H.: Action of carboxypeptidase-A on bovine insulin: Preparation of desalanine-desasparagin-insulin. Biochemistry **2**, 16 (1963).
SOLS, A.: Regulation of liver glucokinase and muscle hexokinase. In: LEIBEL, B.S., and WRENSHALL, G.A., On the nature and treatment of diabetes, p. 118. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.
STEINER, D.F., CLARK, J.L., NOLAN, C., RUBENSTEIN, A.H., MARGOLIASH, E., ATEN, B., OYER, P.E.: Proinsulin and the biosynthesis of insulin. Recent Progr. Hormone Res. **25**, 207 (1969).
— OYER, P.E.: The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc. nat. Acad. Sci. (Wash.) **57**, 473 (1967).
TOMIZAWA, H.H.: Properties of glutathion insulin transhydrogenase from beef liver. J. biol. Chem. **237**, 3393 (1962).
UNGER, R.H., KETTERER, H., DUPRÉ, J., EISENTRAUT, A.M.: The effects of secretin, pancreozymin and gastrin on insulin and glucagon secretion in anaesthetized dogs. J. clin. Invest. **46**, 630 (1967).
WEBER, G., SINGHAL, R.L., SKRIVASTA, S.K.: Insulin: Suppressor of biosynthesis of hepatic gluconeogenic enzymes. Proc. nat. Acad. Sci. (Wash.) **53**, 96 (1965).
WELSH III, G.W., HENLEY, E.D., WILLIAMS, R.H., COX, R.W.: Insulin I^{131} metabolism in man. Amer. J. Med. **21**, 324 (1956).
WOOL, I.G., CAVICCHI, P.: Insulin regulation of protein synthesis by muscle ribosomes: Effect of the hormone on translation of messenger RNA for a regulatory protein. Proc. nat. Acad. Sci. (Wash.) **56**, 991 (1966).
— — Protein synthesis by skeletal muscle ribosomes. Effect of diabetes and insulin. Biochemistry **6**, 1231 (1967).
ZAHN, H.: Struktur und Synthese von Insulin. Verh. dtsch. Ges. inn. Med. **72**, 800 (1967).
ZAHND, G.R., SCHEIDEGGER, J.J.: Rôle de la macro globuline-α_2 du sérum humain dans la fixation in vitro de l'insuline bovine marquée à l'iode^{131}. Helv. med. Acta **30**, 506 (1963).

4. The Acute Metabolic Disturbance in Diabetes and Diabetic Coma

ANDERSON, J.W., KILBOURN, K.J., ROBINSON, J., WRIGHT, P.H.: Diabetic acidosis in rats treated with anti-insulin serum. Clin. Sci. **24**, 417 (1963).
AYDIN, A., SOKAL, J.E.: Uptake of plasma free fatty acids by the isolated rat liver: effect of glucagon. Amer. J. Physiol. **205**, 667 (1963).
BUTLER, A.M.: Medical progress; diabetic coma. New Engl. J. Med. **243**, 648 (1950).
DÉROT, M.: Functional exploration of the insular pancreas. Journées Ann. diabét. Hôtel-Dieu **3**, 101 (1962).
FANKHAUSER, S.: Zur Klinik und Therapie der Insulinresistenz. Praxis **53**, 185 (1964).
FROESCH, E.R.: Fructose metabolism in adipose tissue from normal and diabetic rats. In: A.E. RENOLD and G.F. CAHILL, JR. (eds.), Handbook of physiology, section 5, Adipose tissue, p. 281. Washington: American Physiological Society 1965.
— BÜHLMANN, A., ROSSIER, P.H.: Das Coma diabeticum in heutiger Sicht. Verh. dtsch. Ges. inn. Med. **72**, 199 (1967).
— BÜRGI, H., BALLY, P., LABHART, A.: Insulin inhibition of spontaneous adipose tissue lipolysis and effects upon fructose and glucose metabolism. Molec. Pharmacol. **1**, 280 (1965).
HUCKABEE, W.E.: Abnormal resting blood lactate. I. The significance of hyperlactatemia in hospitalized patients. Amer. J. Med. **30**, 833 (1961).
JOSLIN, E.P., ROOT, H.F., WHITE, P., MARBLE, A.: The treatment of diabetes mellitus, 10. Aufl., p. 461. Philadelphia: Lea & Febiger 1959.
KORNACKER, M.S., LÖWENSTEIN, J.M.: Citrate and conversion of carbohydrate into fat. Biochem. J. **95**, 832 (1965).
LYNEN, F., HENNING, U., BUBLITZ, C., SÖRBO, B., KRÖPLIN-RUEFF, L.: Der chemische Mechanismus der Acetessigsäurebildung in der Leber. Biochem. Z. **330**, 269 (1958).
MACH, R., MACH, E., PLATTNER, H.: Le métabolisme des électrolytes et de l'eau dans le coma diabétique. Schweiz. med. Wschr. **83**, 30 (1953).
NABARRO, J.D.M., BECK, J.C., STOWERS, J.M.: Fructose in treatment of severe diabetic ketosis. Lancet **1955II**, 1271.
PFEIFFER, E.F.: Genesis, diagnosis and treatment of diabetic coma. Internist (Berl.) **1**, 319 (1960).
ROOT, H.F.: Diabetic acidosis and coma. In: JOSLIN, E.P., ROOT, H.F., WHITE, P., and MARBLE, A., The treatment of diabetes mellitus. 10. Aufl., p. 360. Philadelphia: Lea & Febinger 1959.
ROSSIER, P.H.: Études sur l'équilibre acide-base du sang (H. VAILLANT CARMANNE, éd.), p. 97. Liège: Imp. de l'Académie 1932.
— FROESCH, E.R., VÖLLM, K., LABHART, A.: Fortschritte in der Kenntnis der diabetischen Acidose und ihre Konsequenzen für die Therapie. Schweiz. med. Wschr. **90**, 952 (1960).
— REUTTER, F., FRICK, P.: Das hyperosmolare nicht azidotische Koma bei Diabetes mellitus. Dtsch. med. Wschr. **86**, 2145 (1961).
SCHWARTZ, W.B., WATERS, 3rd, W.C.: Lactate versus bicarbonate. A reconsideration of the therapy of metabolic acidosis. Amer. J. Med. **32**, 831 (1962).
STÖTTER, G.: Le coma diabétique. In: Verhdlg. d. 4. Kongr. d. Internat. Diabetes-Gesellsch. Médecine et Hygiène, Genf, 1962, S. 565.
TEUSCHER, A., JEANNERET, P.: Die Therapie der diabetischen Acidose. Medizinische Neuheiten (Mschr. der Fa. Hausmann Ag, St. Gallen) **69**, 63 (1953).
TSCHIRGI, R.D.: Chemical environment of the central nervous system. In: FIELD, J., MASON, H.W., and HALL, V.E. (eds.), Handbook of physiology, section 1, vol. 3, p. 1865. Washington: American Physiological Society 1960.
VAUGHAN, M., STEINBERG, D.: Glyceride biosynthesis, glyceride breakdown and glycogen breakdown in adipose

tissue: mechanisms and regulation. In: RENOLD, A.E., and CAHILL, G.F., JR. (eds.), Handbook of physiology, section 5, Adipose tissue, p. 239. Washington: American Physiological Society 1965.
VÖLLM, K.: Über die Haltbarkeit von Insulin in physiologischer Natriumbicarbonatlösung. Schweiz. med. Wschr. **90**, 1080 (1960).

Pathogenesis and Etiology of Diabetes Mellitus

ANGERVALL, L., SÄVE-SÖDERBERGH, J.: Microangiopathy in the digestive tract in subjects with diabetes of early onset and long duration. Diabetologia **2**, 117 (1966).
ANTONIADES, H.N.: In: B.S. LEIBEL and G.A. WRENSHALL (eds.), The nature and treatment of Diabetes mellitus. Extrapancreatic regulation of insulin activity in human beings, p. 194. Amsterdam: Excerpta Med. Found. 1965.
AZÉRAD, E., LUBETZKI, J., MORARD, J.C., SAMARCQ, P.: Etude en microscopie optique et électronique de 56 fragments de reins diabétiques prélevés par ponction-biopsies: fréquence et précocité des altérations glomérulaires. Diabète (Le Raincy) **12**, 163 (1964).
BLOODWORTH, J.M.B., JR.: Diabetic microangiopathy. Diabetes **12**, 99 (1963).
BUTLER, L.: The inheritance of diabetes in the chinese hamster. Diabetologia **3**, 124 (1967).
BUTTERFIELD, W.J.H., WICHELOW, M.J.: Peripheral glucose metabolism in control subjects and diabetic patients during glucose, glucose-insulin and insulin-sensitivity tests. Diabetologia **1**, 43 (1965).
CAMERINI-DAVALOS, R.A., CAULFIELD, J.B., REES, S.B., LOZANO-CASTANEDA, O., NALDJIAN, S., MARBLE, A.: Preliminary observations on subjects with prediabetes. Diabetes **12**, 508 (1963).
CAMPBELL, G.D.: Diabetes in Asians and Africans in and around Durban. S. Afr. med. J. **37**, 1195 (1963).
CLAWSON, B.J., BELL, E.T.: Incidence of fatal coronary disease in non-diabetic and in diabetic persons. Arch. Path. **48**, 105 (1949).
COHEN, A.M.: Fats and carbohydrates as factors in atherosclerosis and diabetes in Yemenite Jews. Amer. Heart J. **65**, 291 (1963).
COOKE, A.M., FITZGERALD, M.G., MALINS, J.M., PYKE, D.A.: Diabetes in children of diabetic couples. Brit. med. J. **1966II**, 674.
DISCHE, R.M., PAPPAS, G.D., GRAUER, A., DISCHE, Z.: Carbohydrate of basement membranes of human kidney glomeruli. Biochem. biophys. Res. Commun. **20**, 63 (1965).
FIASCHI, E., SCURO, L.A., NACCARATO, R., MONTI, G., SERPI, G., SIVIGU, F.: Intorno alla prime alterazione morfologiche del rene nel diabete giovanile recente. Minerva nefrol. **10**, 95 (1963).
FIELD, J.B., STETTEN, D., JR.: Humoral Insulin antagonism associated with diabetic acidosis. Amer. J. Med. **21**, 339 (1950).
FROESCH, E.R.: Essential fructosuria and hereditary fructose intolerance. In: J.B. STANBURY, J.W. WYNGAARDEN and D.S. FREDRICKSON (eds.), The metabolic basis of inherited disease, p. 124. New York: McGraw Hill 1966.
— BÜRGI, H., MÜLLER, W.A.: Plasmainsulin und Diabetes. Helv. med. Acta **32**, 341 (1965).
— YUDKIN, J. (eds.), Nutrition and Diabetes Mellitus, VIth Capri Conference, Il Ponte, 1972.
FUNK, H.U.: Veränderungen an Pankreaskapillaren bei Diabetikern. Diabetologia **1**, 228 (1966).
GERRITSEN, G.C., DULIN, W.E.: Characterization of diabetes in the Chines hamster. Diabetologia **3**, 74 (1967).
GOETZ, F.C., HARTMANN, J.F., LAZAROW, A.: Electron microscopy of the human glomerulus in early diabetes. J. clin. Invest. **39**, 991 (1960).
GRODSKY, G.M., FELDMAN, R., TORESON, W.W., LEE, J.C.: Diabetes mellitus in rabbits immunized with insulin. Diabetes **15**, 579 (1966).
GROEN, J., GELD, H.V.D., BOLINGER, R.E., WILLEBRANDS, A.F.: The anti-insulin effect of epinephrine. Its significance for the determination of serum insulin by the rat diaphragm method. Diabetes **7**, 272 (1958).
GRÖNBERG, A., LARSSON, T., JUNG, J.: Diabetes in Sweden. A clinico-pathological epidemiological and genetic study of hospital patients and death certificates. Acta med. scand., Suppl. **477** (1968).
HACKEL, D.B., MIKAT, E., LEBOVITZ, H.E., SCHMIDT-NIELSEN, K., HORTON, E.S., KINNEY, T.D.: The sand rat (psammomys obesus) as an experimental animal in studies of diabetes mellitus. Diabetologia **3**, 130 (1967).
HELLMAN, B.: Studies in obese-hyperglycemic mice. Ann. N.Y. Acad. Sci. **131**, 541 (1965).
HOUSSAY, B.A., BIASOTTI, O.A., RIETTI, O.D.: Diabetogenic action of a terior lobe extract. C. R. Soc. Biol. (Paris) **111**, 479 (1932).
— LONG, C.N.H., LUKENS, F.D.E.: The effects of hypophysectomy and adrenalectomy upon diabetes. Trans. Ass. Amer. Physicns **51**, 123 (1936).
JAKOB, A., HAURI, CH., FROESCH, E.R.: Nonsuppressible insulin-like activity in human serum. III. Differentiation of two distinct molecules with nonsuppressible ILA. J. clin. Invest. **47**, 2678 (1968).
KARAM, J.H., GRODSKY, G.M., FORSHAM, P.H.: Excessive insulin response to glucose in obese subjects as measured by immunochemical assay. Diabetes **12**, 197 (1963).
KIMMELSTIEL, P., WILSON, C.: Intercapillary lesions in the glomeruli of the kidney. Amer. J. Path. **12**, 83 (1936).
LECOMPTE, P.M.: "Insulitis" in early juvenile diabetes. Arch. Path. **66**, 450 (1958).
— STEINKE, J., SOELDNER, J.S., RENOLD, A.E.: Changes in the islets of Langerhans in cows injected with heterologous and homologous insulin. Diabetes **15**, 586 (1966).
MACDONALD, M.K., IRELAND, J.T.: The glomerular lesion in idiopathic and secondary diabetes. In: CAMERON, M.P., and O'CONNOR, M., The aetiology of diabetes mellitus and its complications. Ciba Found. Coll. Endocr. **15**, 301 (1964).
MÉRIEL, P., DARNAND, CH., DENGARD, Y., MOREAU, G., SUC, J.M., PUTOIS, J., COMBES, P.F.: La glomérulose diabétique: étude ultra-structurale. Presse méd. **70**, 667 (1962).
MEYENBURG, H. VON: Über „Insulitis" bei Diabetes. Schweiz. med. Wschr. **21**, 554 (1940).
MIALHE, B., SUTTER, CH.J., MEYER, V.: L'adrénaline, inhibiteur de l'insuline sérique chez le rat. Diabetologia **1**, 63 (1965).
MORRISON, A.D., CLEMENTS, R.S., JR., WINEGRAD, A.I.: Effects of elevated glucose concentrations on the metabolism of the aortic Wall. J. clin. Invest. **51**, 3114 (1972).
MUIR, H.: The biochemistry of bloodvessels. In: M.P. CAMERON and O'CONNOR, M.: The aetiology of diabetes mellitus and its complications. Ciba Found. Coll. Endocr. **15**, 282 (1964).
NEEL, J.V.: Diabetes mellitus: a "thrifty" genotype rendered detrimental by progress. Amer. J. hum. Genet. **14**, 353 (1962).
— Proc. Sixth Congress of the Internat. Diabetes Fed., ed. by J. ÖSTMAN. Amsterdam: Excerpta med. Found. 1969.
PARTAMIAN, J.O., BRADLEY, R.F.: Myokardinfarkt bei 258 Diabetikern, unmittelbare Mortalität und 5-Jahres-Überlebensrate. New Engl. J. Med. **273**, 455 (1965).
PICTET, R., ORCI, C., GOUET, A.E., ROUILLER, CH., RENOLD, A.E.: Ultrastructural studies of the hyperplastic islets of Langerhans of spiny mice (Acomys Cahirinus) before and during the development of hyperglycemia. Diabetologia **3**, 188 (1967).
PORTE, D., JR., GRABER, A., KUZUYA, T., WILLIAMS, R.H.: The effect of epinephrine on immunoreactive insulin levels in man. J. clin. Invest. **45**, 228 (1966).
RABINOWITZ, D., ZIERLER, K.L.: Forearm metabolism in obesity and its response to intraarterial insulin. Characterization of insulin resistance and evidence for adaptive hyperinsulinism. J. clin. Invest. **41**, 2173 (1962).

— — Role of free fatty acids in forearm metabolism in man, quantitated by use of insulin. J. clin. Invest. **41**, 2191 (1962).
RENOLD, A.E., STEINKE, J., SOELDNER, J.S., ANTONIADES, H.N., SMITH, R.E.: Immunological response to the prolonged administration of heterologous insulin in cattle. J. clin. Invest. **45**, 702 (1966).
RESKE-NIELSEN, E.K., LUNDBAEK, K., RAFAELSON, O.J.: Pathologic changes in the central and peripheral nervous system of young long-term diabetics. Diabetologia **1**, 233 (1966).
SABOUR, M.S., MACDONALD, M.K., ROBSON, J.S.: An electron microscopic study of the human kidney in young diabetic patients with normal renal function. Diabetes **11**, 291 (1962).
SAMOLS, E.: Immunochemical aspects of insulin. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 247. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.
SÄVE-SÖDERBERGH, J., ANGERVALL, L., FAGERBERG, S.V.: Microangiopathy in young diabetic men. A light microscopic biopsy study of skin with clinical application. Diabetologia **2**, 331 (1966).
SELTZER, H.S., ALLEN, E.W., HERRON, A.L., JR., BRENNAN, M.T.: Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes. J. clin. Invest. **46**, 323 (1967).
SIEVERS, J., BLOMQUIST, G., BIÖRK, G.: Studies in myocardial infarction in Malmö 1935 to 1954. VI. Some clinical data with particular reference to diabetes, menopause and heart rupture. Acta med. scand. **169**, 95 (1961).
STEINBERG, A.G.: Genetics and diabetes. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 601. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.
TAYLOR, K.W., SHELDON, J., PYKE, D.A., OAKLEY, W.G.: Glucose tolerance and serum insulin in the unaffected firstdegree relatives of diabetics. Brit. med. J. **1967**, 4, 22.
VALLENCE-OWEN, J.: Insulin antagonists. In: LEIBEL, B.S., and WRENSHALL, G.A. (eds.), On the nature and treatment of diabetes, p. 340. Amsterdam-New York-London-Milano-Tokyo-Buenos Aires: Excerpta Med. Found. 1965.
WEST, K.M., KALBFLEISCH, J.M., STEIN, J.H.: International epidemiology of diabetes. 5th Congr. int. Diab. Fed. Excerpta med. 1965.
WINEGRAD, A.I., BURDEN, C.L.: L-xylulose metabolism in diabetes mellitus. New Engl. J. Med. **274**, 298 (1966).
WRENSHALL, G.A., BEST, C.H.: Extractable insulin of pancreas and effectiveness of oral hypoglycaemic sulfonylurease in treatment of diabetes in man-comparison. Canad. med. Ass. J. **74**, 968 (1956).
YALOW, R.S., BERSON, S.A.: Plasma insulin concentration in nondiabetic and early diabetic subjects: determinations by a new sensitive immuno-assay technique. Diabetes **9**, 254 (1960).
ZACKS, S.I., PEGUES, J.J., ELLIOTT, F.A.: Internstitial muscle capillaries in patients with diabetes mellitus: A light and electron microscope study. Metabolism **11**, 381 (1962).
ZIERLER, K.L., RABINOWITZ, D.: Roles of insulin and growth hormone, based on studies of forearm metabolism in man. Medicine (Baltimore) **42**, 385 (1964).

Morphology of Diabetes Mellitus

BROLIN, S.E., HELLMAN, B., KNUTSON, H. (edit.): The structure and metabolism of the pancreatic islets. London: Pergamon Press 1964.
CAMERON, M.P., O'CONNOR, M. (edit.): The aetiology of diabetes mellitus and its complications. Ciba Foundation Coll. on Endocrinology, vol. 15. London: Churchill 1964.
COHEN, A.S.: Amyloidosis. New Engl. J. Med. **277**, 522, 574, 628 (1967).
DIEZEL, P.B., WILLERT, H.G.: Morphologie und Histochemie der harten und weichen Exsudate der Retina bei Diabetes mellitus und essentieller Hypertonie. Klin. Mbl. Augenheilk. **139**, 475 (1961).
FISHER, E.R., PEREZ-STABLE, E., AMIDI, M., SARVER, M.E., DANOWSKI, TH.S.: Ultrastructural renal changes in juvenile diabetics. J. Amer. med. Ass. **202**, 291 (1967).
FUNK, H.U.: Veränderungen an Pankreaskapillaren bei Diabetikern. Diabetologia **1**, 228 (1965).
GEPTS, W.: Contribution à l'étude morphologique des ilôts de Langerhans au cours du diabète. Ann. Soc. roy. Sci. méd. nat. Brux. **10**, 5 (1957).
— Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes **14**, 619 (1965).
HEDINGER, CHR.: Zur Pathologie der Haemochromatose als Syndrom. Helv. med. Acta **20**, Suppl. XXXII, 28 (1953).
HELLERSTRÖM, C.: Effects of steroid diabetes on the pancreatic islets of guinea pigs with special reference to the A_1-cells. Acta Soc. Med. upsalien. **68**, 1 (1963).
HELLMAN, B., HELLERSTRÖM, C.: Histology and histophysiology of the islets of Langerhans in man. In: PFEIFFER, E.F. (edit.). Diabetes mellitus, Bd. 1, S. 89. München: J.F.Lehmann 1968.
KIMMELSTIEL, P., OSAWA, G., BERES, J.: Glomerular basement membranes in diabetics. Amer. J. clin. Path. **45**, 21 (1966).
LARSSON, O.: Studies of small vessels in patients with diabetes. Acta med. scand., Suppl. **480** (1967).
LAZARUS, S.S., VOLK, B.W.: The pancreas in human and experimental diabetes. New York-London: Grune & Stratton 1962.
POMETTA, D., ORCI, L., RUFFENER, C., CURCHOD, B.: Etude au microscope électronique des membranes basales des capillaires sanguins musculaires de sujets prédiabétiques, diabétiques et non diabétiques. In press.
RITCHIE, S., WAUGH, D.: The pathology of Armanni-Ebstein diabetic nephropathy. Amer. J. Path. **33**, 1035 (1957).
SEIFERT, G.: Pathologische Morphologie der Langerhansschen Inseln, besonders beim Diabetes mellitus des Menschen. Verh. dtsch. Path. Ges. **42**, 50 (1958).
SELLYEI, M., WALTON, E.: Über Vorkommen und Erscheinungsformen des Kernglykogens in Leberzellen. Zbl. allg. Path. path. Anat. **112**, 1 (1969).
SIEBENMANN, R., STEINER, H., UEHLINGER, E.: In: Handbuch der allgemeinen Pathologie, Bd. VIII/1. Berlin-Heidelberg-New York: Springer, 1972.
SIPERSTEIN, M.D., UNGER, R.H., MADISON, L.L.: Studies on the muscle capillary basement membranes in normal subjects, diabetic and prediabetic patients. J. clin. Invest. **47**, 1973 (1968).
STEINER, H.: Quantitative und qualitative Zellveränderungen im Hypophysenvorderlappen bei Diabetes mellitus. Virchows Arch. path. Anat. **339**, 171 (1965).
WARREN, S., LECOMPTE, PH.M., LEGG, M.A.: The pathology of diabetes mellitus. Philadelphia: Lea & Febiger 1966.
WATTENWYL, N. VON: Neuere Differenzierungsmethoden der Langerhans'schen Inseln. Path. et Microbiol. (Basel) **27**, 144 (1964).

The Clinical Features of Diabetes Mellitus

Reviews, Monographs

BERTRAM, F., OTTO, H.: Die Zuckerkrankheit, 5. Aufl. Stuttgart: G.Thieme 1963.
BOULIN, R.: Traitement du diabète sucré. Paris: G.Doin 1956.

CLEAVE, T.L., CAMPBELL, G.D.: Diabetes, coronary thrombosis and the saccharine disease. Bristol: John Wright 1966.
CONSTAM, G.R.: Therapie des Diabetes mellitus. Basel: Schwabe 1950.
DANOWSKI, T.S. (ed.): Diabetes mellitus with emphasis on children and young adults. Baltimore: Williams & Wilkins 1957.
— Diabetes mellitus, diagnosis and treatment. New York: American Diabetes Ass. 1964.
DEROT, M.: Diabète et Maladies de la Nutrition. Paris: Flammarion 1962.
DUNCAN, G.G.: Diabetes mellitus, principles and treatment. Philadelphia & London: W.B.Saunders 1951.
ELLENBERG, M., RIFKIN, H.: Clinical diabetes mellitus. New York-Toronto-London: McGraw Hill 1962.
JACKSON, W.P.U.: On Diabetes mellitus, selected topics for students and clinicians. Springfield: Ch.C.Thomas 1964.
JOSLIN, E.P., ROOT, H.F., WHITE, P., MARBLE, A.: The Treatment of Diabetes mellitus, 10th ed. Philadelphia: Lea & Febiger 1959.
KUNZ, O.: Das Diabetes-mellitus-Syndrom. München-Berlin: Urban & Schwarzenberg 1965.
LAWRENCE, R.D.: The diabetic life, 17. Aufl. London: J. & A.Churchill 1965.
LISTER, J.: The clinical syndrome of Diabetes mellitus. Springfield: Ch.C.Thomas 1959.
LUEBKEN, W.: Diabetes mellitus, Praxis, Klinik, produktive Fürsorge. Stuttgart: Hippokrates Verlag 1960.
MALINS, J.: Clinical Diabetes mellitus. London: Eyre & Spottiswode 1968.
MARBLE, A., WHITE, P., BRADLEY, R.F., KRALL, L.P.: Joslin's Diabetes mellitus, 11th ed. Philadelphia: Lea & Febinger 1971.
MAURIAC, P.: Retouches au Tableau Classique du Diabète. Paris: Masson 1952.
MOHNIKE, G.: Diabetes mellitus, Theorie-Klinik-Therapie. Berlin: Verlag Volk und Gesundheit 1967.
NUNES, W.T.: Manual of Diabetes for the house officer. Springfield: Ch.C.Thomas 1963.
OAKLEY, W.G., PYKE, D.A., TAYLOR, K.W.: Clinical diabetes and its biochemical basis. Oxford & Edinburgh: Blackwell Scientific Publications 1968.
PALLARDO, L.F.: Diabetes. Pathologia y Clinica. Madrid: Marban 1964.
PATHE, G.: Le Diabète sucré. Paris: Librairie Maloine 1968.
PETRIDES, P., WEISS, L., LÖFFLER, G., WIELAND, O.: Diabetes mellitus. München: Urban & Schwarzenberg 1971.
PFEIFFER, E.F. (Hrsg.): Handbuch des Diabetes mellitus. Band I. Pathophysiologie und Klinik. München: J.F. Lehmann 1969.
ROBBERS, H.: Praktische Diabetologie. München: Werkverlag Dr. Banaschewski 1969.
SCHENCK, E.-G., MELLINGHOFF, C.H.: Der Diabetes mellitus als Volkskrankheit und seine Beziehung zur Ernährung. Darmstadt: Steinkopff 1960.
SEIGE, K.: Diabetes mellitus, 2. Aufl. Leipzig: G.Thieme 1967.
WILDER, R.M.: Clinical diabetes mellitus and hyperinsulinism. Philadelphia: W.B. Saunders 1940.
WILLIAMS, R.H.: Diabetes. New York: Hoeber 1960.

Morbidity

BERTHOLD-GYSI, H.M.: Über die Häufigkeit der Zuckerkrankheit bei Ehepartnern. Praxis **60**, 647 (1971).
BUTTERFIELD, W.J.H.: World Health Organisation. Expert Committee on Diabetes mellitus Draft Agenda item III. 1964.
CLEAVE, T.L., CAMPBELL, G.D.: Diabetes, coronary thrombosis and the saccharine disease. Bristol: John Wright & Sons 1966.
4e Congrès Féd. internat. diabète, Genève 1961, vol. 1, p. 275 (1966).
CONSTAM, G.R.: Volksseuche Diabetes. Ärztl. Fortbild. **16**, 648 (1966).
FISHER, G.F., VAVRA, H.M.: Screening high-yield groups for diabetes. Publ. Hlth Rep. (Wash.) **80**, 961 (1965).
GUENTHER, K.H., KAEDING, A.: Vergleichende Untersuchungen zur Diabeteshäufigkeit bei Nachkommen diabetischer Eltern. 4e Congrès Féd. internat. diabète, Genève 1961, vol. 1, p. 275 (1961).
JACKSON, W.P.U.: On Diabetes mellitus. Springfield: Ch.C. Thomas 1964.
JORDE, R.: The diabetes survey in Bergen, Norway, 1965. Bergen-Oslo: Norwegian Universities Press 1962.
KAEDING, A.: Frühdiagnose der Zuckerkrankheit. Leipzig: G.Thieme 1963.
KHACHADURIAN, A.K., SOMMERVILLE, I.: Diabetes mellitus in Lebanon. J. chron. Dis. **18**, 1309 (1965).
KNOWLES, H.G.: The incidence and development of Diabetes mellitus. In: R.H.WILLIAMS, Diabetes, p. 360. New York: P.B.Hoeber 1960.
KRAINICK, H.G., STRUWE, FR.E.: Zur Situation des kindlichen Diabetes mellitus in West-Deutschland. Dtsch. med. Wschr. **85**, 1632 (1960).
Leading article: Detection of diabetes. Lancet **1963I**, 203.
O'SULLIVAN, J.B., MAHAN, C.M.: Bloodsugar levels, glycosuria and body weight related to development of Diabetes mellitus. The Oxford epidemiologic study 17 years later. J. Amer. med. Ass. **194**, 587 (1965).
OTT, W.: Bevölkerungsbewegung in der Schweiz 1949 bis 56/57. Statistische Quellenwerke d. Schweiz. Heft 275.
RATHERY, M., ROSSELIN, G.: Sur la recherche systématique de la glycosurie chez le personnel d'une banque parisienne. Diabète (Le Raincy) **11**, 163 (1963).
SAGILD, U.H., LITTAUER, C.S., JESPERSEN, ANDERSEN, S.: Diabetes mellitus in Eskimos. Acta med. scand. **179**, 29 (1966).
SCHLENCK, E.G., MELLINGHOFF, C.H.: Der Diabetes mellitus als Volkskrankheit und seine Beziehung zur Ernährung. Darmstadt: D.Steinkopff 1960.
SCHLIACK, V.: Über die Diabetesmorbidität. Dtsch. med. Wschr. **90**, 2321 (1965).
SCHOEFFLING, K.: Früherkennung, Früherfassung und Frühbehandlung der Zuckerkrankheit. Mkurse Ärztl. Fortbild. **15**, 312 (1965).
Statistisch-klinische Diabetesfragen. Leipzig: Akademische Verlagsgesellschaft, Geest & Portig 1953.
TAILLIER, R.: Résultats statistiques de la recherche systématique de l'hyperglycémie alimentaire dans la population de Guéret. Diabète (Le Raincy) **11**, 167 (1963).
TULLOCH, J.A.: The prevalence of Diabetes mellitus in Jamaica. Diabetes **10**, 286 (1961).
US. Public Health Service: Division of chronic disease. Diabetes source book, p. 1168. Washington: Public Health Service Publication, May 1964.
WALKER, J.B., BROWN, P.E.: Early diabetes, a five years follow-up of diabetes in an English community. Lancet **1964II**, 246.
WHO: Expert committee on Diabetes mellitus 1965. WHO. Chron. **20**, 39 (1966).
WILKERSON, H.L.C., KRALL, L.P., BUTLER, F.K.: Diabetes in a New-England Town. J. Amer. med. Ass. **169**, 810 (1959).

Trauma and other Initiating Factors

BRANDY, L.: Disease and injury. Philadelphia-Montreal: Lippincott Co. 1962.
CAMPBELL, G.D., BATCHELOR, L., GOLDBERG, M.D.: Sugar intake and diabetes. Diabetes **16**, 62 (1967).
CLEAVE, T.L., CAMPBELL, G.D.: Diabetes, coronary thrombosis and saccharine disease. Bristol: J.Wright 1966.

FELIX, H., GIROUD, M., QUINOT, B., LACOSTE, J.: Facteurs déclenchants ou révélateurs dans le Diabète de l'Adulte. J. Méd. Lyon **1965**, 1257.
JAHNKE, K., OBERDISSE, K.: Die Begutachtung des Zusammenhangs zwischen Trauma und Diabetes mellitus. Dtsch. med. Wschr. **86**, 2358 (1966).
KANTSCHEN, TH., STAUDACHER, M.: Gutachterliche Probleme der Manifestation oder Verschlimmerung eines Diabetes mellitus nach Trauma, Psychotrauma oder Behandlung mit blutzuckererhöhenden Medikamenten. Acta diabet. lat. **3**, 1 (1966).
KOENIGSTEIN, R.P.: Diabetes mellitus und Saluretika. Stuttgart: G. Thieme 1967.
RANDALL, R.V.: Nonhereditary diabetes mellitus. Proc. Mayo Clinic **41**, 560 (1966).
SCHOEFFLING, K.: Die Begutachtung des Diabetes mellitus. Dtsch. med. Wschr. **91**, 649 (1966).
SEIGE, K., MOHNIKE, G., KOLLMORGEN, G.: Zur einheitlichen Beurteilung des Körperschadens beim Diabetes mellitus. Dtsch. Gesundh.-Wes. **16**, 1327 (1961).
WALES, J.K., GRANT, A., WOLFF, F.W.: Studies on the hyperglycemic effects of nonthiazide diuretics. J. Pharm. experiment. Therap. **159**, 229 (1968).
WASMUTH, K.: Unfall und Zuckerkrankheit. Hefte Unfallheilk. H. 38 (1942).
WHO: Diabetes mellitus. WHO Chron. **20**, 39 (1966).

Prevention

CAMERINI-DAVALOS, R.A.: Prediabetes and the prevention of diabetes. In: DANOWSKI, T.S., Diabetes mellitus, diagnosis and treatment, p. 198. New York: Amer. Diabetes Ass. 1964.
DEUIL, R., LAURENT, CL., MAGDELAINE, M.: Le risque diabétique. Presse méd. **70**, 1879 (1962).
KALK, H.: Über die Beziehungen zwischen Fett, Leber und Diabetes. Dtsch. med. Wschr. **84**, 1898 (1959).
MAURIAC, P.: Retouches au Tableau classique du Diabète. Paris: Masson & Cie. 1952.
PAVEL, I.: La Prophylaxie du Diabète. Presse méd. **70**, 759 (1962).
— PIEPTA, R.: Problèmes que pose l'hérédité diabétique. Journées Ann. Diabète Hôtel Dieu (Paris) **6**, 217 (1965).

Course and Symptomatology

ANGERVALL, L., STENER, B.: Tumoriform focal muscular degeneration in two diabetic patients. Diabetologia **1**, 39 (1965).
CAMERINI-DAVALOS, R.A.: Prediabetes. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment. New York: American Diabetes Assn. 1964.
CONSTAM, G.R.: Heredität und Frühdiagnose. Dtsch. med. Wschr. **89**, 1831 (1964).
DEUIL, R., LAURENT, C.: Hypoglycémie prédiabétique chez trois enfants de mère diabétique. Diabète (Le Raincy) **11**, 241 (1963).
DITSCHUNEIT, H.: Der Prädiabetes. Dtsch. med. Wschr. **90**, 1925 (1965).
PAULSEN, E.P., FELLNER, F.G., LIPIELLO, L.: Carbohydrate tolerance and tolbutamide response in obese children of diabetic families. Diabetes **14**, 438 (1965).
RICKETS, H.T., CHERRY, R.A., KIRSTEINS, L.: Biochemical studies of "prediabetes". Diabetes **15**, 880 (1966).
SCHLIACK, V.: Symptomatik des Diabetes. In: MOHNIKE, Diabetes mellitus. Berlin: Volk & Gesundheit 1967.
SELTZER, H.S., FAJANS, S.S., CONN, J.W.: Spontaneous hypoglycemia as an early manifestation of Diabetes mellitus. Diabetes **5**, 437 (1956).
WHO: Diabetes mellitus. WHO Chron. **20**, 39 (1966).
WILANSKY, D.L., SOCHAT, G.: The course of latent diabetes. Conference on Diabetes mellitus and obesity. New York 1967.

Differential Diagnosis

ANDRES, R., LANGS, H., POZEFSKY, T., SWERDLOFF, R., GREGERMANN, R.J.: Alterations in carbohydrate metabolism with age. 7th International congress of gerontology, Vienna, 1966. Proceedings. Wien: Wiener med. Akad. 1966.
BERNARD, C.: Leçons de physiologie expérimentale appliquée à la médecine. Paris: Baillière 1855.
CHLOUVERAKIS, C., JARRET, R.J., KEEN, H.: Glucose tolerance, age and circulating insulin. Lancet **1967 I**, 806.
CLEMENT, R.: Intolérance au saccharose. Presse méd. **75**, 969 (1967).
CONSTAM, G.R.: Differential diagnosis of Diabetes mellitus. Northw. Med. (Seattle) **1957**, 919.
— Über die Differentialdiagnose des Diabetes mellitus. Schweiz. med. Wschr. **90**, 333 (1960).
— Melliturias other than Diabetes mellitus. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment. New York: American Diab. Assn. 1964.
— HEGGLIN, R.: Frühbehandlung und Frühdiagnose des Diabetes mellitus. Schweiz. med. Wschr. **94**, 1284 (1964).
— ISLER, K.M.: Über eine einfache Methode zur Differenzierung der verschiedenen Zucker im Harn. Gastroenterologia (Basel) **87**, 145 (1957).
FALUDI, G., BENDERSKY, G.: Treatment of functional hypoglycemia in early latent diabetes. Conference on Diabetes mellitus and Obesity. New York 1967.
FANCONI, G.: Der nephrotisch-glykosurische Zwergwuchs mit hypophosphatämischer Rachitis. Jahrb. Kinderheilk. **147**, 299 (1963).
FIELD, R.A.: Glycogen deposition diseases. In: STANBURY-WYNGAARDEN-FREDRICKSON, The metabolic basis of inherited diseases, 2nd ed. New York: MacGraw Hill 1966.
FROESCH, E.R.: Essential fructosuria and hereditary fructose intolerance. In: STANBURY-WYNGAARDEN-FREDRICKSON, The metabolic basis of inherited disease, 2nd ed. New York: McGraw Hill 1966.
HIATT, H.H.: Pentosuria. In: STANBURY-WYNGAARDEN-FREDRICKSON, The metabolic basis of inherited disease, 2nd ed. New York: MacGraw Hill 1966.
ISSELBACHER, K.J.: Galactosemia. In: STANBURY-WYNGAARDEN-FREDRICKSON, The metabolic basis of inherited disease, 2nd ed. New York: MacGraw Hill 1966.
KNOWLES, H.C.: Syrupy blood. Diabetes **15**, 760 (1966).
KOPECKY, A., KELLEN, J.: Papierchromatographie der Zukker in der Klinik. Leipzig: G. Thieme 1963.
KRANE, S.M.: Renal glycosuria. In: STANBURY-WYNGAARDEN-FREDRICKSON, The metabolic basis of inherited disease. 2nd ed. New York: MacGraw Hill 1966.
LAWRENCE, R.: The diabetic life, 17. Aufl. London: J.A. Churchill 1965.
LEAF, A.: The syndrome of osteomalacia, renal glycosuria, aminoaciduria and increased phosphorus clearance (The Fanconi syndrome). In: STANBURY-WYNGAARDEN-FREDRICKSON, The metabolic basis of inherited disease, 2nd ed. New York: McGraw Hill 1966.
MUETING, D.: Früherkennung des Diabetes mellitus in Praxis und Klinik. Med. Welt **1966**, 1733.
PERELMANN, R., LESTRADET, H.: Diabète néonatal. Ann. Pédiat. **40**, 283 (1964).
REUBI, F.: Recherches sur le diabète rénal. Helv. med. Acta **18**, 69 (1951).
ROBBERS, H.: Der renale Diabetes. Stuttgart: Wissenschaftl. Verlagsgesellschaft 1946.
SCHREIER, K.: Nichtdiabetische Melliturien. Ergebn. inn. Med. Kinderheilk. **12**, 493 (1959).
SCRIBA, P.C., SCHWARZ, K., HOFMANN, G.G.: Vergleich klinischer Methoden zur Erfassung des latenten Diabetes. Dtsch. med. Wschr. **91**, 753 (1966).
TONI, G.: Remarks on relation between renal rickets and renal diabetes. Acta paediat. (Stockh.) **16**, 479 (1953).
TRAISMAN, H.S., NEWCOMB, A.L.: Management of juvenile diabetes mellitus. St. Louis: C.V. Mosby 1965.

WESTERVELT, F.B., SCHREINER, G.E.: The carbohydrate intolerance of uremic patients. Ann. intern. Med. **57**, 266 (1962).
WHO: Diabetes mellitus. WHO Chron. **20**, 39 (1966).

Prognosis

BELL, E.T.: Postmortem study of 1214 diabetic subjects with special reference to the vascular lesions. Proc. Amer. Diabetes Ass. **10**, 62 (1950).
CAIRD, F.I., PIRIE, A., RAMSELL, T.G.: Diabetes and the eye. Oxford and Edinburgh: Blackwell Sci. Publ. 1969.
CONSTAM, G.R.: La déception du diabète. Méd. Hyg. **21**, 522 (1963).
— Zur Spätdiagnose des Diabetes mellitus. Helv. med. Acta **32**, 287 (1965).
— The long-term prognosis of diabetes mellitus. Ann. Life Insur. Med. **3**, 229 (1967).
— Die Ergebnisse der pädiatrischen Behandlung aus der Sicht des Internisten. Suppl. zu Arch. Kinderheilk. **58**, 37 (1968).
— The long-term prognosis of Diabetes mellitus—a statistical follow-up of cases. Life Ass. Med. Proc. 10th internat. confer. life assur. med. 115 (1970).
— Prognosestellung bei Zuckerkranken aus versicherungsmedizinischer Sicht. Lebensversicher.-Med. **23**, 90 (1971).
— REICH, TH.: Ist die „freie" oder „normale" Kost in der Behandlung des Zuckerkranken harmlos? Schweiz. med. Wschr. **90**, 14 (1960).
ENTMACHER, P.S., MARKS, H.H.: Diabetes in 1964. A world survey. Diabetes **14**, 212 (1965).
— — ROOT, H.F.: Longevity of diabetic patients in recent years. Diabetes **13**, 373 (1964).
FAJANS, S.S., CONN, J.W.: Praediabetes, subclinical diabetes and latent clinical diabetes. In: LEIBEL and WRENSHALL, Nature and treatment of diabetes, p. 641. New York: Excerpta Medica Foundation 1965.
KRAINICK, H.G., STRUWE, TH.: Zur Situation des kindlichen Diabetes mellitus in West-Deutschland. Dtsch. med. Wschr. **85**, 1632 (1960).
MALINS, J.M.: Causes of death in diabetics. In: L.J.P. DUNCAN, Diabetes mellitus, p. 137. Edinburgh: University Press 1966.
MARKS, H.H.: Longevity and mortality of diabetics. Amer. J. publ. Hlth **55**, 416 (1965).
MEISSNER, W.A., LEGG, A.M.: The pathology of diabetes in Joslin's Diabetes mellitus, 11th ed. Philadelphia: Lea & Febiger 1971.
MOSS, W.T.: Common pecularities of patients with adenocarcinoma of endometrium; with special reference to obesity, body build, diabetes and hypertension. Amer. J. Roentgenol. **58**, 203 (1947).
OAKLEY, W.C., PYKE, D.A., TAYLOR, K.W.: Clinical diabetes. Oxford and Edinburgh: Blackwell Sci. Publ. 1968.
PLAUCHU, M., DELMAIS, J., PINET, G., TETE, M., BRYON, P.-A., BOURLIER: Les causes de la mort de 100 diabétiques adultes. Diabète (Le Raincy) **10**, 1 (1962).
ROHRACHER, T.: Spätschicksale zuckerkranker Kinder. Wien: Wilhelm Maudrich 1951.
ROOT, H.F.: Prognosis in idiopathic diabetes mellitus. In: WILLIAMS, R.H., Diabetes, p. 642. New York: P.B. Hoeber 1960.
ROSENBUSCH, H.: Prognose und Spätkomplikationen des Diabetes mellitus im Kindesalter. Basel: S. Karger 1945.
SEIGE, K.: Zur sozialmedizinischen Bedeutung des Diabetes mellitus. In: Diabetes mellitus, 3. Congress International Diabetes Federation, Düsseldorf 1958. Hrsg. K. OBERDISSE, u. K. JAHNKE, S. 681. Stuttgart: G. Thieme 1959.
WARREN, S.W., LE COMPTE, PH.M.: The pathology of diabetes. In: JOSLIN et al., Treatment of Diabetes mellitus, 10. Aufl., p. 188. Philadelphia: Lea & Febiger 1959.
World Health Organisation: Annual epidemiological and vital statistics 1958, 1959, 1960. Geneva, WHO, 1961 to 1963.

Aim and Principles of the Treatment of Diabetes

CONSTAM, G.R.: Zur Frage der Diabetiker-Ambulatorien. In: OBERDISSE u. JAHNKE, Fortschritte der Diabetesforschung, S. 97. Stuttgart: G. Thieme 1963.
— Langzeittherapie des Diabetes mellitus. Hippokrates (Stuttg.) 1967.
HARDIN, R.C.: Goals. In: DANOWSKI, T.S., Diabetes mellitus, diagnosis and treatment, p. 65. New York: American Diabetes Ass. 1964.
MARBLE, A.: Criteria of control. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment, p. 69. New York: American Diabetes Ass. 1964.
RICKETS, H.T.: Diabetes mellitus, objectives and methods of treatment. Springfield: Ch.C. Thomas 1955.

Dietary Treatment

ANTAR, M.A., OHLSON, M.A.: Effects of simple and complex carbohydrates upon total lipids, nonphospholipids and different fractions of phospholipids of serum in young men and women. J. Nutr. **85**, 329 (1965).
ARNOULD, Y., BALASSE, E., FRANCKSON, J.R.M., BASTENIE, P.A.: Effect of protracted starvation on the glycoregulation of obese patients. Diabetologia **1**, 136 (1965).
BOUR, H., DEROT, M.: Guide pratique de Diététique. Paris: Baillière & fils 1966.
CONSTAM, G.R.: Leitfaden für Zuckerkranke, 8. Aufl. Basel: Schwabe & Co. 1970.
— Nahrungsmitteltabellen für Zuckerkranke, 8. Aufl. Basel/Stuttgart: Schwabe & Co. 1970.
— Grundlagen und Kritik der diätetischen Empfehlungen beim Diabetes mellitus der Erwachsenen. Klinische Ernährungslehre III, p. 8. Darmstadt: Steinkopff 1970.
CUBBERLEY, P.T., POLSTER, S.A., SCHULLMAN, C.L.: Lactic acidosis and death after the treatment of obesity by fasting. New Engl. J. Med. **272**, 628 (1965).
Deutsches Diabetes-Komitee: Kohlenhydrat-Austausch-Tabelle, 4. Aufl. Stuttgart: G. Thieme 1966.
ESTRICH, D., RAVNIK, A., SCHLIERF, G., FUKUYAMA, G., KINSELL, L.: Effects of co-ingestion of fat and protein upon carbohydrate-induced hyperglycemia. Diabetes **16**, 232 (1967).
— — — — KINSELL, L.W.: Long-acting carbohydrate. Diabetes **15**, 527 (1966).
HARRISON, M.T., HARVEN, R.M.: The longterm value of fasting in the treatment of obesity. Lancet **1966II**, 1340.
LINQUETTE, M., FOSSATI, P., LEFEBRE, J., FOURLINNIE, J.-C., DECOULX, M.: Etude du métabolisme glucidique au cours de 33 cures de jeûne total chez 30 obèses. Diabète (Le Raincy) **15**, 103 (1967).
ROYER, P., LESTRADET, H.: Traitement du diabète infantile en régime libre. Paris: Flammarion 1958.
SCHLESS, G.L., DUNCAN, G.G.: The beneficial effect of intermittent total fasts on the glucose tolerance in obese diabetic patients. Metabolism **15**, 98 (1966).
SIMONIN, R., ROUX, H., CALOTTY, G., VAGUE, J.: Inégalité des obèses devant les régimes isocaloriques mais de composition différente. Diabète (Le Raincy) **13**, 1 (1965).
YUDKIN, J., CAREY, M.: The treatment of obesity by the "high-fat" diet, the evitability of calories. Lancet **1960II**, 939.

Treatment with Sulfonylurea-Derivatives

AYLETT, P.: Effects of tolbutamide on gastric secretion and emptying. Brit. med. J. **1965I**, 1464.
BENNER, E.J., PARTRIDGE, J.W., HOLCOMB, B.: An evaluation of long-term chlorpropamide therapy. J. Amer. med. Ass. **193**, 763 (1965).

BERGER, W., CONSTAM, G.R.: Perorale Behandlung des Diabetes mellitus mit Medikamenten von verschiedenem Wirkungsmechanismus. Schweiz. med. Wschr. **97**, 444 (1967).

BLOODWORTH, J.M.B.: Morphologic changes associated with sulfonylurea therapy. Metabolism **12**, 287 (1963).

BUNNAG, S.C., WARNER, N.E., BUNNAG, S.: Effect of tolbutamide on postnatal neogenesis of the islands on Langerhans in mouse. Diabetes **15**, 5 (1966).

BURKE, G., SILVERSTEIN, G.E., SORKIN, A.I.: Effect of long-term sulfonylurea therapy on thyroid function in man. Metabolism **16**, 651 (1967).

BUTTERFIELD, W.J.H., WESTERING, W. VAN: Tolbutamide... after ten years. Brook Lodge Symposium 1967. Amsterdam: Excerpta med. Foundation 1967.

CHOI, Y., KREINES, K.: The use of glycodiazine in adult-onset diabetes. Diabetes **15**, 530 (1966).

CHU, P.-C., CONWAY, M.J., KROUSE. H.A., GOODNER, CH.J.: The pattern of response of plasma insulin and glucose to meals and fasting during chlorpropamide therapy. Ann. intern. Med. **68**, 757 (1968).

CONSTAM, G.R.: Sulfonylurea compounds in the treatment of diabetics. Northw. Med. (Seattle) **56**, 1155 (1957).

— Is the use of sulphonylurea derivatives in the treatment of Diabetes mellitus dangerous? Diabetologia **7**, 237 (1971).

CREUTZFELDT, W., SOELING, H.D.: Orale Diabetestherapie und ihre experimentellen Grundlagen. Ergebn. inn. Med. Kinderheilk. **15**, 1 (1960).

DUBACH, U.C., BUCKERT, A., RAAFLAUB, J.: Einfluß von Sulfonamiden auf die blutzuckersenkende Wirkung oraler Antidiabetica. Schweiz. med. Wschr. **96**, 1483 (1966).

FAJANS, S.S., CONN, J.W.: The use of tolbutamide in the treatment of young people with mild diabetes mellitus. A progress report. Diabetes **11**, Suppl. 123 (1962).

FIELD, J.B., OHTA, M., BOYLE, C., REMER, A.: Potentiation of acetohexamid hypoglycemia by butazolidin. Diabetes **15**, 520 (1966).

GERRITSEN, G.C., DOOLIN, W.E.: Effect of a new hypoglycemic agent 3,5-dimethylpyrazole on carbohydrate and free fatty acid metabolism. Diabetes **14**, 507 (1965).

HALLER, H., STRAUZENBERG, ST.E.: Perorale Diabetestherapie. Leipzig: G.Thieme 1959.

HASSELBLATT, A.: Biochemische Gesichtspunkte zur Erklärung der Blutzucker-senkenden und antidiabetischen Wirksamkeit von Sulfonylharnstoff-Derivaten. Internist (Berl.) **7**, 369 (1966).

HEINKINHEIMO, R.: Severe prolonged hypoglycemia following tolbutamide and carbutamide treatment. Diabetes **14**, 606 (1965).

KERP, L., KASEMIR, H., KIELING, F.: Insulin bindende Antikörper und Insulinbedarf bei Diabetikern. Klin. Wschr. **46**, 376 (1968).

KNICK, B.: Erfahrungen und Kritik der Sulfonylharnstofftherapie extradiabetischer Indikationen. Verh. dtsch. Ges. inn. Med. **67**, 160 (1961).

— LANGE, H.-J., BAIER, H., JOCKE, H., HAAS-SCHEUREN, G.: Zur Problematik der exocrinen Pankreasreaktion nach intravenöser Tolbutamid-Belastung bei Stoffwechselgesunden und Diabetikern. Klin. Wschr. **42**, 507 (1964).

KRISTENSEN, M., HANSEN, J.M.: Potentiation of the tolbutamide effect by dicoumarol. Diabetes **16**, 211 (1967).

LOUBATIERES, A.: Analyses de l'action antidiabétique des sulfamides hypoglycémiants, fondement de son utilisation pour déterminer la réduction durable ou la prévention du diabète. Presse méd. **68**, 1441 (1960).

MEHNERT, H., REISNER, E.: Studien zum Problem des sog. Spätversagens der Sulfonylharnstofftherapie. Dtsch. med. Wschr. **89**, 1378 (1964).

RULL, J.A., LENNHOFF, M.: Prolonged and recurrent tolazamide-induced hypoglycemia. Diabetes **16**, 352 (1967).

SAMOLS, E., TYLER, J.M., MIALHE, P.: Suppression of pancreatic glucagon release by hypoglycemic sulphonylureas. Lancet **1969I**, 174.

SCHLESS, G.L., LEE, C.T., JR.: Oral hypoglycemic therapy and associated hypothyroidism. Conference on Diabetes mellitus and Obesity. New York 1967.

SCHULZ, E., BRINKMANN, W.: Rezidivierendes hypoglykämisches Koma infolge Abbaustörung von Tolbutamid. Dtsch. med. Wschr. **93**, 485 (1968).

STOWERS, J.M., HELGASON, E., HELGASON, TH.: Trial of chlorpropamide in subclinical diabetes. Diabetologia **1**, 128 (1965).

STUART, R.D., ANDERSEN, D.E.: Glycodiazine in diabetes mellitus. Brit. med. J. **1965II**, 682.

YOSHINAGA, T., YAMAMOTO, Y.: Über Beziehungen zwischen Sulfonylharnstoffen und einigen Metall-Ionen. Endokrinologie **50**, 87 (1966).

Treatment with Biguanides

BECKMANN, R.: Zum Wirkungsmechanismus der Biguanide. Dtsch. med. Wschr. **90**, 1598 (1965).

BERGER, W., CONSTAM, G.R.: Perorale Behandlung des Diabetes mellitus mit Medikamenten von verschiedenem Wirkungsmechanismus. Schweiz. med. Wschr. **97**, 444 (1967).

— — SIEGENTHALER, W.: Die Behandlungsmöglichkeiten des Diabetes mellitus mit Biguaniden. Schweiz. med. Wschr. **96**, 1335 (1966).

BRUNS, W.: Klinische Anwendung von Guanidinderivaten. In: MOHNIKE, Diabetes mellitus, S.199. Berlin: Verlag Volk u. Gesundheit 1966.

CZYZYK, A., LAWECKI, J.: Untersuchungen über den Einfluß von Phenylaethylbiguanid auf den Verlauf von Belastungsproben mit Insulin, Tolbutamid und Glucose bei Diabetes mellitus. Diabetologia **2**, 62 (1966).

DAVIDOFF, F.F.: Oral hypoglycemic agents and the mechanism of Diabetes mellitus. New Engl. J. med. **278**, 148 (1968).

GOODMANN, J.I.: Role of phenformin (DBI) as an adjuvant in oral antidiabetic therapy. Metabolism **14**, 1153 (1965).

LIPPMANN, H.G.: Guanidinderivate und deren Wirkungsmechanismus. In: MOHNIKE, Diabetes mellitus, S.185. Berlin: Verlag Volk u. Gesundheit 1966.

MEHNERT, H.: Experimentelle und klinische Untersuchungen zum Wirkungsmechanismus und Indikationsbereich blutzuckersenkender Biguanidderivate. München: A. Schubert 1963.

PFEIFFER, E.F.: Die orale Behandlung des Diabetes mellitus. Bayerische Landesärztekammer Schriftenreihe **3**, 40 (1964).

SAUER, H.: Die Biguanidbehandlung des Diabetes mellitus. Fortschr. Med. **79**, 605 (1961).

SEARLE, G.L., SCHILLING, S., PORTE, D., BARBACCIA, J., GRAZIA, J. DE, CAVALIERI, R.R.: Body glucose kinetics in non-diabetic human subjects after phenethylbiguanid. Diabetes **15**, 173 (1966).

Treatment with Insulin

ABEL, J.J.: Crystalline insulin. Proc. nat. Acad. Sci. (Wash.) **12**, 122 (1926).

ADAMS, M.J., BLUNDELL, T.L., DODSON, E.J., DODSON, G.G., VIJAYAN, M., BAKER, E.N., HARDING, M.M., HODGKIN, D.G., RIMMER, B.B., SHEAT, S.: Structure of rhombohedral 2 zinc insulin crystals. Nature (Lond.) **224**, 491 (1969).

ARNOTT, J.H., LITTLE, J.A.: Sulfated insulin in mild, moderate, severe and insulin resistant diabetes mellitus. Diabetes **14**, 440 (1965).

AUBERTIN, E., TEXIER, L., AUBERTIN, J., AUGARDE, D.: Sur 30 cas de lipodystrophies insuliniques. J. Méd. Bordeaux **142**, 605 (1965).
BERGER, S., VONGARAYA, N.: Insulin response to ingested protein in diabetics. Diabetes **14**, 442 (1965).
BOLLINGER, R.E., MORRIS, J.H., MCKNIGHT, F.G., DIEDERICH, D.D.: Disappearance of I^{131} labeled insulin from plasma as a guide to management of diabetes. New York med. J. **270**, 767 (1964).
BUTTERFIELD, W.J.H., ABRAMS, M.E., SELL, R.A., STERKY, S.G., WICHELOW, J.M.: Insulin sensitivity of the human brain. Lancet **1966I**, 557.
CONSTAM, G.R.: Übersicht über die verschiedenen Insulinarten und ihren Indikationsbereich in der Behandlung des Diabetes mellitus. Schweiz. med. Wschr. **77**, 715 (1947); Las diversas insulinas y sus indicaciones en el tratamento de la diabetes sacarina. Día méd. **20**, 1158 (1948).
— Neuerungen in der Insulinbehandlung. Helv. med. Acta **16**, 406 (1949).
— Über ein modifiziertes Protamin-Zink-Insulin, das sogenannte NPH-Insulin. Helv. med. Acta **18**, 494 (1951).
— Erfahrungen mit Insulin-Zink-Suspensionen, einer Gruppe von Insulinpräparaten (Lente Insuline) mit verschieden langer Wirkungsdauer. Schweiz. med. Wschr. **84**, 200 (1954).
— Leitfaden für Zuckerkranke, 8. Aufl. Basel: Schwabe & Co. 1970.
— Thrombocytopenic purpura as a probable manifestation of insulin allergy. Diabetes **5**, 121 (1956).
— ALLAN, F.N.: A case of hemochromatosis refractory to insulin. Proc. Mayo Clin. **3**, 225 (1928).
DAWEKE, H.: Klinik der Insulinresistenz. Dtsch. med. Wschr. **91**, 973 (1966).
DECKERT, T.: Insulinantistoffer. Nord. Med. **73**, 288 (1965).
ENSINCK, J.W., POFFENBARGER, L., HOGAN, R.A., WILLIAMS, R.H.: Studies of insulin antagonism. Diabetes **16**, 289 (1967).
EZRIN, C., MOLONEY, P.J.: Resistance to insulin due to neutralizing antibodies. J. clin. Endocr. **19**, 1055 (1959).
FANKHAUSER, S., DIACON, CH.: Die Bedeutung der Insulin-Antikörper bei Komplikationen der Insulintherapie. Helv. med. Acta, Suppl. **46**, 164 (1966).
FEDERLIN, K., HEINEMANN, G., GIGLI, I., DITSCHUNEIT, H.: Antigenbindung durch zirkulierende Leucozyten bei der verzögerten lokalen Insulinallergie. Dtsch. med. Wschr. **91**, 814 (1966).
FIELD, J.B.: Chronic insulin-resistant diabetics. Geriatrics **18**, 846 (1963).
HALLAS-MOELLER, K.: Chemical and biological insulin studies. Kopenhagen: Arnold Busck 1945.
JUNG, H.D., GLOCKNER: Multiple lokalisierte Hautknochenbildungen und -verkalkungen an Insulin-Injektionsstellen. Dtsch. med. Wschr. **91**, 1974 (1966).
KATSOYANNIS, P.G.: Symposium on insulin. Amer. J. Med. **40**, 652 (1966).
KUHNLEIN, E.: Hypoglykämische Dauerschäden bei der Insulinbehandlung. In: OBERDISSE u. JAHNKE, Diabetes mellitus, S. 733. Stuttgart: G. Thieme 1959.
LACEY, A.H.: The unit of insulin. Diabetes **16**, 198 (1967).
LAZAROW, A.: Insulin synthesis, storage, release, transport and antagonism. Diabetes **15**, 281 (1966).
LENHARDT, A.: Versuch des Selbstmordes mittels massiver Insulindosen. Wien. med. Wschr. **114**, 808 (1964).
LITTLE, J.A., ARNOTT, J.H.: Sulfated insulin in mild, moderate, severe and insulinresistant diabetes mellitus. Diabetes **15**, 457 (1966).
MEYTHALER, F., KOTLORZ, H.: Insulinresistenz. Ärztliche Forsch. **19**, H. 5 (1965).
MUCCI, A., GORI, E.C., ZANDOMENEGHI, R.: Correlazioni fra alcune funzioni psichoneurologiche e livelli glicemici. Acta diabet. lat. **3**, 351 (1966).
NIELSEN, A.V., BINDER, C.: The absorption of soluble insulin correlated to the diabetic state. Acta med. scand. **181**, 521 (1967).
NORA, J.J., SMITH, D.W., CAMERON, J.R.: The route of insulin administration in the management of diabetes mellitus. J. Pédiat. **64**, 547 (1964).
OAKLEY, W.G.: Insulin-resistance. Brit. med. J. **1967II**, 134.
PFEIFFER, E.F.: Die Insulinresistenz. Dtsch. med. Wschr. **91**, 314 (1966).
— Die Immunologie des Insulins. Verh. dtsch. Ges. inn. Med. **72**, 811 (1966).
SHIPP, J.C., CUNNINGHAM, R.W., RUSSEL, R.O., MARBLE, A.: Insulin resistance. Medicine (Baltimore) **44**, 165 (1965).
TUCKER, W.R., KLINK, D., GOETZ, F., ZALME, E., KNOWLES, H.: Insulinresistance and acanthosis nigricans. Diabetes **13**, 395 (1964).

Accompanying Symptoms and Complications of Diabetes Mellitus

ALEXANDER, K., NISSEN, P., MITZKAT, H.J., HARTMANN, F.: Der angiologische Status eines ambulanten diabetischen Krankengutes. Dtsch. Arch. klin. Med. **213**, 173 (1967).
ANGERVALL, L., SAVE-SOEDERBERG, J.: Microangiopathy in the digestive tract in subjects with diabetes of early onset and long duration. Diabetologia **2**, 117 (1966).
BECKER, D., MILLER, M.: Presence of diabetic glomerulosclerosis in patients with hemochromatosis. New Engl. J. Med. **263**, 367 (1960).
BLUMENTHAL, H.T., GOLDBERG, S., BERNS, A.W.: Pathology and pathogenesis of the disseminated angiopathy of diabetes mellitus. In: LEIBEL and WRENSHALL, Nature and treatment of Diabetes mellitus, p. 397. New York: Excerpta medica Foundation 1965.
BURTON, T.Y., KERNS, T.P., RYNEARSON, E.: Diabetic retinopathy following total pancreatectomy. Proc. Mayo Clin. **32**, 735 (1957).
DITZEL, J.: Functional microangiopathy in diabetes mellitus. Diabetes **17**, 388 (1968).
— WHITE, P., DUCKERS, J.: Changes in the pattern of the smaller blood vessels in the bulbar conjunctiva in children of diabetic mothers. Diabetes **3**, 99 (1954).
DOYLE, A.P., BALCERZAK, S.P., JEFFREY, W.L.: Fetal diabetic glomerulosclerosis after total pancreatectomy. New Engl. J. Med. **270**, 623 (1964).
Editorial: L'angiopathie diabétique. Presse méd. **74**, 1931 (1966).
EWING, C., ZORILLA, E., HERNANDEZ, A., BERRIS, R.: Diabetic vascular complications in longstanding panhypopituitarism with diabetes. Diabetes **15**, 419 (1966).
GEILER, G.: Die diabetische Angiopathie aus der Sicht des Pathologen. Dtsch. med. Wschr. **92**, 1640 (1967).
GOLDENBERG, S., BLUMENTHAL, H.T.: Artherosclerosis. In: DANOWSKI, S.T., Diabetes mellitus, diagnosis and treatment. New York: American Diab. Assn. 1964.
HEVELKE, A.: Angiochemische Untersuchungen über die Vorzeitigkeit der Altersveränderungen diabetischer Gefäße. In: BUERGER, Angiopathia diabetica, S. 11. Stuttgart: G. Thieme 1954.
KAEDING, A.: Diabetes-Komplikationen. Stuttgart: Ferdinand Enke 1956.
LUNDBAEK, K.: Late developments in long-term diabetic vascular disease. In: OBERDISSE u. JAHNKE, Diabetes mellitus, S. 141. Stuttgart: G. Thieme 1959.
MOHNIKE, G.: Diabetische Angiopathie. Berlin: Akademie Verlag 1964.
PATZ, A., BERKOW, J.W., MAUMENSEE, A.E., COX, J.: Studies on diabetic retinopathy, retinopathy and nephropathy in spontaneous canine diabetes. Diabetes **14**, 700 (1965).
SÄVE-SOEDERBERG, J., ANGERVALL, L., FAGERBERG, S.-E.: Microangiopathy in young diabetic men. Diabetologia **3**, 331 (1967).

Changes in the Eyes

BLOODWORTH, J. M. B.: Pathogenesis of diabetic retinopathy. In: L. J. P. DUNCAN, Diabetes mellitus, p. 75. Edinburgh: University Press 1966.

BORN, J. L., LAWRENCE, J. H., LINFOOT, J. A., TOBIA, C. A., MANOUGIAN, E., SNYDER, N. J.: Hypophyseal suppression with heavy particles in diabetic retinopathy. In: LEIBEL and WRENSHALL, Nature and treatment of Diabetes mellitus, p. 475. New York: Excerpta medica Foundation 1965.

CAIRD, F. I.: Prognosis for vision in diabetic retinopathy in LEIBEL and WRENSHALL, Nature and Treatment of Diabetes mellitus, p. 465. New York: Excerpta medica Foundation 1965.

— PIRIE, A., RAMSELL, T. G.: Diabetes and the eye. Oxford and Edinburgh: Blackwell Sci. Publ. 1969.

COLWELL, J. A.: Effect of diabetic control on retinopathy. Diabetes **15**, 497 (1966).

CONSTAM, G. R.: Diabetes mellitus und seine Beziehungen zur Retinopathie. Ophthalmologica (Basel) **135**, 436 (1958).

DECKERT, T., SIMONSEN, S. E., POULSEN, J. E.: Prognosis of proliferative retinopathy in juvenile diabetics. Diabetes **16**, 728 (1967).

DITZEL, J., WHITE, P., DICKER, J.: Changes in the pattern of the smaller blood vessels in the bulbar conjunctiva in children of diabetic mothers. Diabetes **3**, 104 (1954).

DOLENEK, A., TAKAC, A.: Atlas der Retinopathia diabetica. Stuttgart: G. Fischer 1966.

ERNEST, I., LINNER, E., SVANBORG, A.: Carbohydrate rich, fat poor diet in diabetes. Amer. J. Med. **39**, 594 (1965).

FRASER, R.: Treatment of retinopathy. In: L. J. P. DUNCAN, Diabetes mellitus (treatment of retinopathy by pituitary ablation), p. 177. Edinburgh: University Press 1966.

FUTTERWEIT, W., SCHWARTZ, M. J., MIRSKY: Blepharoptosis in diabetes mellitus. Diabetes **14**, 455 (1965).

JAEGER, E.: Beiträge zur Pathologie des Auges. Wien 1855.

JOPLIN, G. F., OAKLEY, N. W., HILL, D. W., KOHNER, E. M., FRASER, T. R.: Diabetic retinopathy. II. Comparison of disease remission induced by various degrees of pituitary ablation by Y^{90}. Diabetologia **3**, 406 (1967).

KIMURA, S. J., CAYGILL, W. M.: Vascular complications of diabetes mellitus with special emphasis on microangiopathy of the eye. Saint Louis: C. V. Mosby 1967.

LARSEN, H.-W.: Atlas of diabetic retinopathy. Rep. Steno Hosp. (Kbh.) **8**, 1 (1959).

— X-ray therapy in proliferative diabetic retinopathy. Acta ophthal. (Kbh.) **37**, 531 (1959).

— Diabetic retinopathy. Copenhagen I. A. Diss. 1960.

LAWRENCE, J. H., TOBIAS, C. A., BORN, J. L., LINFOOT, J. A., D'ANGIO, G. J.: Heavy particles in experimental medicine and therapy. J. Amer. med. Ass. **196**, 166 (1966).

Leading Article: Natural History of Diabetic Retinopathy. Lancet **1968II**, 391.

LENTI, G., TOSELLI, C., SIRGU, F., SERRA, A., PELLEGRINI, A.: Rilievi di microscopia ottica ed elettronica sui capillari congiuntivali in corso di malattia diabetica. Acta diabet. lat. **3**, 342 (1966).

LUFT, R., OLIVERCRONA, H., IKKOS, D., KORNERUP, T., JUNGGREN, H. L.: Hypophysectomy in man. Brit. med. J. **1955II**, 752.

PATZ, A.: Diabetic blindness. Arch. Ophthal. **75**, 455 (1966).

POULSEN, J. E.: The Houssay phenomenon in man, recovery from retinopathy in a case of diabetes with Simmond's disease. Diabetes **2**, 7 (1953).

— Diabetes and anterior pituitary insufficiency. Diabetes **15**, 73 (1966).

POWELL, E. D. U., FIELD, R. A.: Studies on salicylates and complement in diabetes. Diabetes **15**, 730 (1966).

— FRANTZ, A. G., RABKIN, M. T., FIELD, R. A.: Growth hormone in relation to diabetic retinopathy. New Engl. J. Med. **275**, 922 (1966).

RUCKER, C. W., GASTINEAU, C. F., SVIEN, H. J.: Effect of section of pituitary stalk on diabetic retinopathy. Proc. Mayo Clin. **42**, 409 (1967).

SAFIR, A., PAULSEN, E. P., KLAYMAN, J., GERSTENFELD, J.: Ocular abnormalities in juvenile diabetics. Arch. Ophthal. **76**, 557 (1966).

SCHRADER, K. E., MAHR, G., NEUMANN, T.: Zur Therapie der Retinopathia diabetica mit Fructose. Dtsch. med. Wschr. **91**, 306 (1966).

TEUSCHER, A.: Die Hypophysenausschaltung zur Behandlung der diabetischen Retinopathie. Auswahl der Patienten für eine Hypophysektomie. Praxis **55**, 1350 (1966).

— FANKHAUSER, S., GIRARD, J., WYSS, F.: The significance of endocrine activity in hypophysectomized diabetics. Helv. med. Acta **33**, 29 (1966).

THIEL, R.: Der Diabetes mellitus ein Gefäß-Problem? Büch. Augenarzt No. 25 (1956).

WHITTINGTON, T, H.: Retinal angiopathy in diabetes. Diabetologia **4**, 176 (1968).

YANOFF, M.: Diabetic retinopathy. New Engl. J. Med. **274**, 1344 (1966).

Diseases of the Nervous system

AXELSSON, A., FAGERBERG, S.-E.: Auditory function in diabetics. Acta oto-laryng. (Stockh.) **66**, 49 (1968).

BISCHOFF: Die diabetische Neuropathie. Stuttgart: G. Thieme 1963.

— LUETHY: Betrachtungen zur pathologischen Anatomie motorischer Lähmungen bei Diabetes mellitus. Dtsch. Z. Nervenheilk. **185**, 252 (1963).

BLEULER, M.: Endokrinologische Psychiatrie. Stuttgart: G. Thieme 1954.

BLOODWORTH, J. M. B., EPSTEIN, M.: Diabetic amyotrophy. Diabetes **16**, 181 (1967).

BOULIN, R., LAPRESLE, J., GUENIOT, M.: Etudes de cerveau des diabétiques. 2. Kongr. intern. Diabet. Fed. Cambridge 1954.

DIETERLE, P.: Die diabetische Anhidrose. Dtsch. med. Wschr. **92**, 1159 (1967).

Editorial: Altered nerve metabolism in diabetes. Diabetes **15**, 696 (1966).

ELLENBERG, M.: Diabetic neuropathy of upper extremities. Diabetes **14**, 454 (1967).

FAGERBERG, S.: Recent advances in diabetic neuropathy. In: LEIBEL and WRENSHALL, Nature and treatment of Diabetes mellitus, p. 522. New York: Excerpta med. Found. 1965.

GABBAY, K. H., O'SULLIVAN, J. B.: The sorbitol pathway. Enzyme localization and content in normal and diabetic nerve and cord. Diabetes **17**, 239 (1968).

GAMSTORP, I., SHELBURNE, S. A., ENGLESON, G., REDONO, D., TRAISMAN, H. S.: Peripheral neuropathy in juvenile diabetes. Diabetes **15**, 411 (1965).

GOTTSTEIN, U., HELD, K.: Insulinwirkung auf den menschlichen Hirnmetabolismus von Stoffwechselgesunden und Diabetikern. Klin. Wschr. **45**, 18 (1967).

GREGERSEN, G.: Variations in the motor conduction velocity produced by acute changes of the metabolic state in diabetic patients. Diabetologia **4**, 273 (1968).

GREGORY, R. F., MILIS, G. C., GREGORY, R. L.: Cerebrospinal fluid lipids in diabetes mellitus. Diabetes **15**, 471 (1966).

JORGENSEN, M. B., BUCH, N. H.: Studies of the sense of smell and taste in diabetics. Acta oto-laryng. (Stockh.) **53**, 539 (1961).

KISSEL, P., DEBRY, G., DUC, M., VAILLIANT, G., DUC, M.-L.: Le reflèxogramme achillèen chez les diabétiques. Presse méd. **74**, 11 (1966).

KORP, W., LEVETT, R. E., SUMMER, K.: Klinik und Verlauf der Polyneuropathie beim jugendlichen Insulinmangeldiabetes. Verh. dtsch. Ges. inn. Med. **73**, 1165 (1966).

LUNDBAEK, K.: Long-term Diabetes. Copenhagen: E. Munksgard 1953.

MARIGO, S., FERRERO, E.: Il trattamento di una grave neuropathia diabetica con insuline sommistrate per via endorachidea. Acta diabet. lat. **1**, 276 (1964).
MONTENERO, P.: Paraparésie spastique comme première manifestation clinique du diabète. Diabète **12**, 85 (1964).
NOELLE, H.: Die Korrelation von neurologischen Krankheitsbildern zu latentem und manifestem Diabetes mellitus. Verh. dtsch. Ges. inn. Med. **73**, 1162 (1966).
PIRART, J.: Le diabète entraine-t-il des dommages au niveau de l'appareil cochléovestibulaire? Acta oto-rhino-laryng. belg. **15**, 469 (1961).
PUFF, K.H.: Die sogenannte diabetische Amyotrophie. Dtsch. med. Wschr. **87**, 255 (1962).
RAFF, M.C., SANGALANG, V., ASHBURY, A.K.: Ischemic mononeuropathy multiplex associated with diabetes mellitus. Arch. Neurol. Psychiat. (Chic.) **18**, 487 (1968).
RESK-NIELSEN, LUNDBAEK, K., RAFAELSEN, O.J.: Pathological changes in the central and peripheral nervous system of young long-term diabetics. Diabetologia **1**, 233 (1965).
SCHELLING, J.-L., TETREAULT, L., LASAGNA, L., DAVIS, M.: Abnormal taste threshold in diabetes. Lancet **1965I**, 508.
SIEVERS, P.: Gastrektasie bei diabetischer Neuropathie. Dtsch. Z. Verdau.- u. Stoffwechselkr. **26**, 218 (1966).
VERNER, J.W., MORRISON, A.M.: Islet cell tumor and a syndrome of refractory watery diarrhea and hypocalcemia. Amer. J. Med. **25**, 374 (1958).

Disorders of the Cardiovascular System

ALBRINK, M.J., LAVIETES, P.H., MAN, E.B.: Vascular disease and serum lipids in diabetes mellitus. Observation over 30 years. Ann. intern. Med. **58**, 305 (1963).
ALEX, M., BARON, E., GOLDENBERG, S., BLUMENTHAL, H.T.: An autopsy study of cerebral vascular accident in diabetes mellitus. Circulation **25**, 663 (1962).
BANSE, H.J., ZAHNOW, W.: Neue medikamentöse Behandlungsmöglichkeiten der Angiopathie bei Diabetikern. Münch. med. Wschr. **100**, 2036 (1958).
BANSON, B.B., LACY, P.E.: Diabetic microangiopathy in human toes. Amer. J. Path. **45**, 41 (1964).
BELL, E.T.A.: Post-mortem study of vascular disease in diabetes. Arch. Path. **53**, 444 (1952).
BENCOSME, S.A., WEST, R.O., KERR, J.W., WILSON, D.O.: Diabetic capillary angiopathy in human skeletal muscles. Amer. J. Med. **40**, 67 (1966).
KAPPERT, A.: Der akute Arterienverschluß der Extremitäten. Bern: Hans Huber 1960.
KNIKER, W.T.: Immunogenic vasculitis, is it part of diabetes mellitus? In: KIMURA and CAYGILL, Vascular complications of Diabetes mellitus, p. 210. Saint Louis: C.V. Mosby 1967.
KOENIGSTEIN, R. P.: Diabetes mellltus and Saluretika. Stuttgart: G. Thieme 1967.
KREMER, G.J., NIEMCZYK, H., KNICK, B.: Coronarsklerose und subklinischer Diabetes mellitus. Schweiz. med. Wschr. **97**, 735 (1967).
LEDET, TH.: Histological and histochemical changes in the corornary arteries of old diabetic patients. Diabetologia **4**, 268 (1968).
LIPPMANN, H.I.: Physiatrists role in the care of diabetes. N.Y. St.J. Med. **66**, 1649 (1966).
MARTI, W.K.: Intraarterielle Langzeitinfusionen mit Vasodilatatoren und niedrigmolekularem Dextran in der Behandlung peripherer arterieller Durchblutungsstörungen. Schweiz. med. Wschr. **93**, 1746 (1963).
MCKITTRICK, J.B.: Surgical procedures. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment. New York: Amer. Diab. Assn. 1964.
MCKITTRICK, L.S., MCKITTRICK, J.B., RISLEY, TH.S.: Transmetatarsal amputation for infection or gangrene in patients with diabetes mellitus. Ann. Surg. **130**, 826 (1949).
PAUL, T.N.: Treatment by local application of insulin of an infected wound in a diabetic. Lancet **1966II**, 574.
RATSCHOW, M.: Angiologie. Stuttgart: G. Thieme 1959.
SCHOOP, W.: Angiologie-Fibel. Stuttgart: G. Thieme 1964.
— MARTIN, M., ZEITLER, E.: Beseitigung aller Arterienverschlüsse durch intravenöse Streptokinaseinfusion. Dtsch. med. Wschr. **93**, 2321 (1968).
WARENBOURG, H., JAILLARD, J.: Retentissement métabolique et hémodynamique du diabète sur le myocarde. Journées Ann. Diabète Hôtel Dieu. Paris **6**, 195 (1965).
WIDMER, L.K.: Der chronische Verschluß von Gliedmaßenarterien. Dtsch. med. Wschr. **91**, 863 (1966).
WHITEHOUSE, F.W., JURGENSEN. C., BLOCK, M.A.: The Later Life of the Diabetic Amputee. Diabetes **17**, 520 (1968).

Diseases of the Kidneys and the Urinary Tract

BELL, E.T.: Renal vascular disease in diabetes mellitus. Diabetes **2**, 388 (1953).
BRIGGS, J.D., BUCHANAN, K.D., LUKE, R.G., MCKIDDIE, M.T.: Role of insulin in glucose intolerance in uremies. Lancet **1967I**, 462.
CERLETTI, J.M., ENGEBRING, N.H.: Azotemia and glucose intolerance. Ann. intern. Med. **66**, 1097 (1967).
DITZEL, J., SCHWARZ, M.: Abnormally increased glomerular filtration rate in short term insulin treated diabetic subjects. Diabetes **16**, 264 (1967).
ELLENBERG, M.: Diabetic neurogenic vesical dysfunction. Arch. intern. Med. **117**, 348 (1966).
FABRE, J., JEANDET, J., CHATELANAT, F.: Etude anatomoclinique des pyélonéphrites et néphrites interstitielles. Berne: Hallwag 1964.
FARQUHAR, M.G.: Fine structure of normal and diabetic glomerular capillaries. In: KIMURA and CAYGILL, Vascular complications of Diabetes mellitus, p. 99. Saint Louis: C.V. Mosby 1967.
GIBB, B., GIBELMANN, G., MARTIN, S., MARTIN, F., SCHULZ, M., LIPPMANN, H.G.: Nachweis von Haptoglobinen und Isoagglutininen im Harn bei diabetischer Angiopathie. Diabetologia **1**, 219 (1965).
HEUCHEL, G.: Klinische Statistik der diabetischen Nephropathie. Jena: G. Fischer 1961.
HUTCHINGS, R.H., HEGSTROM, R.M., SCRIBNER, B.H.: Glucose intolerance in patients on long-term intermittent dialysis. Ann. intern. Med. **65**, 275 (1966).
KIMMELSTIEL, P.: Basement membrane in diabetic glomerulosclerosis. Diabetes **15**, 61 (1966).
— OSAWA, G., BERES, J.: Glomerular basement membrane in diabetics. Amer. J. Path. **45**, 21 (1966).
LECOMPTE, PH.: Glomerulosclerosis. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment. New York: Amer. Diab. Assn. 1964.
LUNDBAEK, K.: Nephropathy in diabetic subjects. In: LEIBEL and WRENSHALL, Nature and treatment of Diabetes mellitus, p. 436. New York: Excerpta Med. Found. 1965.
PANZRAM, G., ANGER, G., THIELER, H., WOELLNER, H.: Verstärkte physiologische Proteinurie als Frühsymptom der diabetischen Nephropathie. Dtsch. Gesundh.-Wes. **21**, 610 (1966).
RIFKIN, H., LEITER, L., BERKMAN, J.: Diabetic glomerulsclerosis. Springfield: Ch.C. Thomas 1952.
SCHEITLIN, W.: Diabetische Nephropathie. Praxis **55**, 1331 (1966).
— LABHART, A.: Die diabetische Nephropathie. In: Handbuch der inneren Medizin, Bd. VIII. 5. Auflage. Berlin-Heidelberg-New York: Springer 1968.
SYLLABA, J., OPPELT, J.: Classification du syndrome de Kimmelstiel-Wilson basé sur des expériences cliniques et des recherches biochimiques. 4. Kongress Internat. Diab. Fed., p. 513. Genf 1961.
TCHOBROUTSKY, G., COLLIN DE L'HORTET, G., ROSSELIN, G., ASSAN, R., DEROT, M.: Etude de la glycorégulation dans l'insuffisance rénale chronique. Diabetologia **1**, 101 (1965).
THOMSEN, Å.CHR.: The kidney in Diabetes mellitus. Copenhagen: Munksgaard 1965.

Infections

BOSHELL, B. R.: Infection. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment. New York: Amer. Diab. Assn. 1964.

BYBEE, J. D., ROGERS, D. E.: The phagocytic activity of polymorphonuclear leucocytes obtained from patients with diabetes mellitus. J. Lab. clin. Med. **64**, 1 (1964).

GRACIANSKY, P. DE: Les infections cutanées chez le diabétique. Journées Ann. Diabète Hôtel Dieu Paris **7**, 164 (1966).

GRAFE, E.: Diabetes und Tuberkulose. Stuttgart: G. Thieme 1948.

LAWONN, H.: Lungentuberkulose bei Diabetes. In: MOHNIKE, G., Diabetes mellitus, S. 235. Berlin: Volk und Gesundheit 1967.

MASKE, H.: Über die Herabsetzung der Penicillinwirksamkeit durch Diabetiker-Serum. Verh. dtsch. Ges. inn. Med. **58**, 99 (1952).

MULLER-WIELAND, K.: Besonderheiten der Pathogenese und Symptomatologie der Diabetiker-Tuberkulose. Dtsch. med. Wschr. **85**, 1650 (1960).

SMITH, J. A., O'CONNOR, J., WILLIS, A. T.: Nasal carriage of staphylococcus aureus in diabetes mellitus. Lancet **1966 II**, 776.

Skin Changes

BAUER, M. F., HIRSCH, P., BULLOTZ, W. K., ABUL-HAJ, S. K.: Necrobiosis lipoidica. Arch. Derm. **90**, 558 (1964).

BINKLEY, G. W.: Dermopathy in the diabetic syndrome. Arch. Derm. **92**, 625 (1966).

CANTWELL, A. R., MARTZ, W.: Idiopathic bullae in diabetics. Arch. Derm. **96**, 42 (1967).

CIVATTE, J.: Xanthomes des diabétiques. Journées Ann. Diabète Hôtel Dieu Paris **7**, 159 (1966).

COTTENOT, F.: Histochimie de la peau diabétique. Journées Ann. Diabète Hôtel Dieu Paris **7**, 161 (1966).

DEGOS, R.: Nécrobiose lipoïdique des diabétiques. Journées Ann. Diabète Hôtel Dieu Paris **7**, 147 (1966).

LAYMON, C. W., HILL, E. M.: An appraisal of hyalinosis cutis et mucosae. Arch. Derm. **95**, 55 (1967).

LUNDBAEK, K.: Late developments in long-term diabetic vascular disease. In: OBERDISSE and JAHNKE, Diabetes mellitus, p. 141. Stuttgart: G. Thieme 1959.

MELIN, H.: An atrophic circumscribed skin lesion in the lower extremities of diabetics. Acta med. scand., Suppl. **423** (1964).

MONTENERO, P., COLLETTI, A., FABRI, G.: Maladie de Dupuytren et diabète. Journées Ann. Diabét. Hôtel Dieu Paris **6**, 75 (1965).

MÜLLER, S. A.: Dermatologic disorders associated with diabetes mellitus. Proc. Mayo Clin. **41**, 689 (1966).

— WINKELMANN, R. K.: Necrobiosis lipoidica diabeticorum. J. Amer. med. Ass. **195**, 433 (1966).

MURPHY, R. A.: Skin lesions in diabetic patients: the "spotted-leg" syndrome. Lahey Clin. Found. Bull. **14**, 10 (1965).

SPRING, M., COHEN, B. D.: Dupuytren's contraction a warning of diabetes? Diabetes **15**, 547 (1966).

THANNHAUSER, S. J.: Werner's syndrome (Progeria of the adult) and Rothmund's syndrome: two types of closely related heredofamilial atrophic dermatosis with juvenile cataracts and endocrine features. Ann. intern. Med. **23**, 559 (1945).

Diseases of the Digestive System, the Exocrine Pancreas and the Liver

ANGERVALL, L., SAVE-SOEDERBERGH, J.: Microangiopathy in the digestive tracts in subjects with diabetes of early onset and long duration. Diabetologia **2**, 117 (1966).

BAIER, H., ROTHER, F., BAUER, H., KNICK, B.: Leberbioptische Befunde bei verschiedenen Formen von Diabetes mellitus. Münch. med. Wschr. **17**, 89 (1965).

BELL, E. T.: Carcinoma of the pancreas. Amer. J. Path. **33**, 499 (1957).

BLOODWORTH, J. M. B.: Diabetes mellitus and cirrhosis of the liver. Arch. intern. Med. **108**, 695 (1961).

BRAUNSTEINER, H., DI PAULI, R., SAILER, S., SANDHOFER, F.: Cholelithiasis und latent diabetische Stoffwechsellage. Schweiz. med. Wschr. **96**, 44 (1966).

CANIVET, J., BATTESTI, J. P.: Le diabète des affections pancréatiques. Diabète (Le Raincy) **9**, 191 (1961).

CHEY, W. Y., SHAY, H., SHUMAN, CH. R.: External pancreatic secretion in diabetes mellitus. Ann. intern. Med. **59**, 812 (1963).

CREUTZFELDT, W.: Klinische Beziehungen zwischen Diabetes mellitus und Leber. Acta hepatosplenol. **6**, 156 (1959).

DAVIS, R. A., BROOKS, F. P., ROBERT, C.: Gastric secretory response to graded insulin hypoglycemia. Amer. J. Physiol. **208**, 6 (1965).

DOTEWALL, G.: Gastric emptying in diabetes mellitus. Acta med. scand. **170**, 423 (1961).

FELBER, J.-P., MAGNENANT, P., VANNOTTI, A.: Tolérance au glucose diminuée et réponse insulinique elevée dans la cirrhose. Schweiz. med. Wschr. **97**, 1537 (1967).

FELDMANN, M., FELDMAN, M., JR.: The incidence of cholelithiasis, cholesterosis and liver disease in diabetes mellitus. Diabetes **3**, 305 (1954).

FINESTONE, A. J., BOORUIY, S. R.: Diabetes mellitus and periodontal disease. Diabetes **16**, 336 (1967).

FINLAY, J. M.: Gastrointestinal complications of diabetes. In: LEIBEL and WRENSHALL: Nature and treatment of diabetes mellitus, p. 563. New York: Excerpta med. Found. 1965.

HANEFELD, M., NAUMANN, H. J., HALLER, H.: Statistische Untersuchungen über den Einfluß des Diabetestyps und der Therapieform auf die Ausprägung und Frequenz der Leberverfettung bei Diabetes mellitus. Z. Verdau.- u. Stoffwechselkr. **27**, 13 (1967).

HENSLEY, G. T., SOERGEL, K. H.: Neuropathologic findings in diabetic diarrhea. Arch. Path. **85**, 587 (1968).

HOLT, J. M.: Solitary pyogenic liver abscess in patients with diabetes mellitus. Lancet **1966 II**, 198.

KALK, H.: Über die Beziehungen zwischen Fett, Leber, und Diabetes. Dtsch. med. Wschr. **84**, 1898 (1959).

KASSANDER, P.: Asymptomatic gastric retention in diabetics. Ann. intern. Med. **48**, 797 (1958).

KNICK, B.: Primary hepatic steatosis as a metabolic change in early diabetes. Acta diabet. lat. **3**, 224 (1966).

KRENTZ, K.: Funktionelle und histo-pathologische Befunde der Corpus-Schleimhaut des Magens bei Zuckerkranken. Diabetologia **2**, 205 (1966).

LACY, P. E.: Quantitative histochemistry of the islets of Langerhans. Diabetes **11**, 96 (1962).

LARCAN, A., HURIET, C., CHARLES, J.-M.: La gastroparésie des diabétiques. Diabète (Le Raincy) **14**, 13 (1966).

LAWRENCE, R. D.: Evanescent parotitis in diabetes. Brit. med. J. **1965 II**, 1432.

LEGER, L., CHICHE, P., FOURE, J., LEMAIGRE, G., Pancréatites chroniques et artérites. Presse méd. **71**, 1017 (1963).

MARKS, I. N., SCHUMAN, CH. R., SHAY, H.: Gastric acid secretion in diabetes mellitus. Ann. intern. Med. **51**, 227 (1959).

MEGYESI, C., SAMOLS, E., MARKS, V.: Glucose tolerance and diabetes in chronic liver disease. Lancet **1967 II**, 1051.

MIDDLETON, G. D., HOCKADAY, T. D. R Glycogen-loaden hepatomegaly in diabetes. Diabetologia **1**, 116 (1965).

MIDTGAARD, K.: Diabetic visceral neuropathy. Diabetes **15**, 93 (1966).

MIROUZE, J.: Le foie diabétique, aspects hormonaux. Paris: Expansion 1951.
MOHNIKE, G., ULRICH, K.H.: Erkrankungen der Zähne und ihres Halteapparates bei Diabetikern. Klin. Wschr. **1954**, 432.
PARMA, C.: Paradentosis diabetica. Paradontologia **1**, 5 (1947).
PIRART, J.: Diabète et appareil digestif. Acta gastro-ent. belg. **30**, 343 (1967).
RITTER, U.: Die excretorische Pankreas-Sekretion bei Diabetes mellitus. Dtsch. med. Wschr. **90**, 1063 (1965).
SHANNON, I.L., O'LEARY, T.J., GIBSON, W.A.: Oral glucose tolerance and periodontal status. Acta diabet. lat. **3**, 33 (1966).
SHUMAKER, H.B.: Acute pancreatitis and diabetes. Ann. Surg. **112**, 177 (1940).
SPRAGUE, R.G.: Diabetes mellitus associated with chronic relapsing pancreatitis. Proc. Mayo Clin. **22**, 533 (1947).
ULRICH, K.H.: Stomatologische Beobachtungen bei Diabetikern. Halle: C.Marhold 1954.
WEGMANN, T., SOLLBERGER, W.: Die symmetrische asymptomatische Parotishypertrophie, Symptom einer chronischen Pankreasinsuffizienz. Schweiz. med. Wschr. **90**, 508 (1960).

Diseases of the Sexual Organs

BATAILLE, J.P.: Le testicule du diabétique impuissant. Diabète (Le Raincy) **11**, 290 (1963).
BUCHLER, D., WARREN, J.C.: Effects of estrogen on glucose tolerance. Amer. J. Obstet. Gynec. **95**, 479 (1966).
ELLENBERG, M., WEBER, H.: Retrograde ejaculation in diabetic neuropathy. Ann. intern. Med. **65**, 1237 (1966).
FEDERLIN, K., SCHOEFFLING, K., NEUBRONNER, P., PFEIFFER, E.F.: Histometrische Untersuchungen am Hodengewebe des Diabetikers mit Keimdrüsenunterfunktion. Diabetologia **1**, 85 (1965).
FREYCHET, P., ROSSELIN, G.E., ASSAN, R., TCHOBROUTSKY, D., DOLAIS, J., DEROT, M.: Un cas de diabète sucré associé à dysgénésie gonadique. Etude radio-immunologique plasmatique des hormones somatotropes, folliculo-stimulante et thyréotrope. Presse méd. **75**, 2381 (1967).
KLEBANOW, D., MACLEOD, J.: Semen quality and certain disturbances of reproduction in diabetic man. Fertil. and Steril. **11**, 255 (1960).
NORRIS, H.J., YUNIS, E.: Age changes of seminal vesicules and vasa deferentia in diabetes. Arch. Path. **77**, 126 (1964).
PETERSON, W.F., STEEL, M.W., JR., COYNE, R.V.: Analysis of the effect of ovulatory suppressants on glucose tolerance. Amer. J. Obstet. Gynec. **95**, 484 (1966).
PLUM, F.: The neurologic complications of diabetes mellitus. In: WILLIAMS, Diabetes. New York: Paul B.Hoeber 1960.
POST, R.H.: Early menarchial age of diabetic women. Diabetes **11**, 287 (1962).
RUBIN, A., BABBOTT, D.: Impotence and diabetes mellitus. J. Amer. med. Ass. **168**, 498 (1958).
SCHOEFLING, K.: Störungen der Keimdrüsenfunktionen bei männlichen Zuckerkranken. Stuttgart: F.Enke 1960.
— Hypogonadism in male diabetic subjects. In: LEIBEL and WRENSHALL, Nature and treatment of Diabetes mellitus, p. 505. New York: Excerpta med. Found. 1965.
SILAGY, J.M.: Urogenital complications in diabetes. In: ELLENBERG and RIFKIN, Clinical diabetes, p. 337. New York: McGraw-Hill Book Co. 1962.
SPALLACY, W.N., CARLSON, K.L.: Plasma insulin and blood glucose levels in patients taking oral contraceptives. Amer. J. Obstet. Gynec. **95**, 474 (1966).
WYNN, V., DOAR, J.W.: Some effects of oral contraceptives on carbohydrate metabolism. Lancet **1966II**, 715.

Diseases of the Hypophysis (see p. 124)

Diseases of the Adrenal Glands (see p. 393)

Diseases of the Pitiurity (see p. 246, 265)

Diseases of the Blood and of Hemopoiesis

ABASTADO, M., DOUMENC, J., ROST, R., CASUBOLO, L., SAMAMA, M., DEROT, M., BOUSSER, J.: Exploration des activités fibrolynitiques et plaquettaires chez le diabétique. Diabète (Le Raincy) **15**, 119 (1967).
ANDERSEN, J., LAURITZEN, E.: Blood groups and diabetes mellitus. Diabetes **9**, 20 (1960).
ARAPAKIS, G., BOCK, O.A., WILLIAMS, D.L., WITTS, L.J.: Diabetes mellitus and pernicious anemia. Brit. med. J. **1963I**, 159.
BERG, K., AARSETH, S., LUNDEVALL, J., REINSKOU, T.: Blood groups and genetic serumtypes in diabetes mellitus. Diabetologia **3**, 30 (1967).
BRIDGES, J.M., DALBY, A.M., MILLER, J.H., BEAVER, J.: An effect of D-glucose on platelet stickiness. Lancet **1965I**, 75.
BUCKWALTER, J.A.: Diabetes mellitus and the blood groups. Diabetes **13**, 164 (1964).
CONSTAM, G.R.: Thrombocytopenic purpura as a probable manifestation of insulin allergy. Diabetes **5**, 121 (1956).
GASSMANN, W., SCHNEEWEIS, J.: Diabetes mellitus and pernicious anemia. Acta haemat. (Basel) **16**, 396 (1956).
INTROZZI, P., NICOLA, P. DE: Thrombophilia in diabetes: thrombelastographic investigations. Diabetes **6**, 433 (1957).
MAYNE, E.E., WEAVER, J.A., BRIDGES, J.M.: A study of platelet adhesion in diabetes mellitus. Diabetologia **4**, 377 (1968).
MOORE, J.M., NELSON, J.M.: Antibodies to gastric mucosa and thyroid in diabetes mellitus. Lancet **1963II**, 645.
SKOVBORG, F., NIELSON, A.V., SCHLICHTKRULL, J., DITZEL, J.: Blood viscosity in diabetic patients. Lancet **1966I**, 129.
UNGAR, B., STOCKS, E., WITTINGHAM, F.I.R., MACKAY, I.R.: Intrinsic-factor antibody in diabetes mellitus. Lancet **1967II**, 77.
WEINBERG, F.S.: Diabetes mellitus and pernicious anemia. Amer. J. dig. Dis. **17**, 398 (1951).

Diseases of the Bones and Joints

AZERAD, E., LUBETZKI, J., STUHL, L., SLOTINE, M.: Les ostéopathies du diabète sucré. Ouest méd. **17**, 529 (1964).
BAILEY, C.C., ROOT, H.F.: Neuropathic foot lesion in diabetes mellitus. New Engl. J. Med. **236**, 397 (1947).
BEIDLEMAN, B., DUNCAN, G.G.: Charcot joints and infectious vascular lesions of bones in diabetes mellitus. Amer. J. Med. **12**, 43 (1952).
BOEHM, H.J.: Diabetic Charcot joint. New Engl. J. Med. **267**, 185 (1962).
BOULET, P.: Le pied diabétique. Journées Ann. Diabét. Hôtel Dieu Paris **2**, 41 (1961).
DARNAUD, CH., DENARD, Y., FOURNIE, A., AYROLLES, CH.: Arthropathie neurologique du pied au cours d'une polynévrite diabétique. Diabète (Le Raincy) **15**, 127 (1967).
DEROT, M., CORMIER, J.M., MOREL-MAROGER, A., RATHERY, M.: Fractures spontanées chez les diabétiques. Diabète (Le Raincy) **10**, 82 (1962).
— RATHERY, M.: Pied cubique diabétique et syndrome du scaphoide tarsien. Journées Ann. Diabét. Hôtel Dieu Paris **8**, 285 (1967).
— — Le syndrome du scaphoide tarsien. Diabète (Le Raincy) **15**, 131 (1967).
FREHNER, H.-U., HOHL, K.: Diabetes und Wirbelsäule. Helv. med. Acta **28**, 502 (1961).

FROST, H.M., TAKAHASHI, H.: Decreased cortical bone resorption in ribs of patients with well-controlled diabetes mellitus. Diabetes **14**, 454 (1965).
HEIDELMANN, G.: Sudeck-Syndrom als Folge obliterierender Angio-Organopathien. Verh. dtsch. Ges. inn. Med. **64**, 323 (1958).
HERNBERG, C.A.: Skelettveränderungen bei Diabetes mellitus des Erwachsenen. Acta med. scand. **143**, 1 (1952).
HEUCK, F., SCHMIDT, E.: Zur Osteoporose bei Diabetes mellitus. Verh. dtsch. Ges. inn. Med. **62**, 464 (1956).
JULKUNEN, H., KARAVA, R., VILJANEN, V.: Hyperostosis of the spine in Diabetes mellitus and acromegaly. Diabetologia **2**, 123 (1966).
KUHLENCORDT, F., WIENERS, H., GOCKE, H.: Skelettuntersuchungen bei Diabetikern bis zum 45. Lebensjahr. Dtsch. med. Wschr. **91**, 1913 (1966).
MARTIN, M.M.: Charcot joints in diabetes mellitus. Proc. roy. Soc. Med. **45**, 503 (1952).
MICHON, P., MICHON, J., LARCAN, A., ROUILLARD, J., LECLERE, J., ROSENZWEIG, O.: Les ostéo-arthropathies diabétiques. Diabète (Le Raincy) **2**, 253 (1961).
MIROUZE, J.: Ostéose Pagétique et diabète sucré. Sem. Hôp. Paris **31**, 837 (1955).
MOHNIKE, G.: Beiträge zum Problem der Osteoporose. Beitr. Orthop. Traum. **8**, 665 (1961).
OTT, V.R., SCHWENKENBECHER, H., ISER, H.: Die Spondylose bei Diabetes mellitus. Z. Rheumaforsch. **22**, 278 (1963).
PARSONS, H., NORTON, W.S.: Management of diabetic neuropathic joints. New Engl. J. Med. **244**, 935 (1951).
ROBILLARD, R., GAGNON, P.-A., ALARIE, R.: Les neuroarthropathies diabétiques. Un. méd. Can. **93**, 1051 (1964).

Tumors

Annotations: Diabetes and endometrial cancer. Lancet **1961 I**, 496.
BELL, E.T.: Carcinoma of the pancreas. Amer. J. Path. **33**, 499 (1957).
COHEN, G.F.: Early diagnosis of pancreatic neoplasma in diabetics. Lancet **1965 II**, 267.
CONSTAM, G.R.: In: Therapie des Diabetes mellitus, S. 250. Basel: Schwabe 1950.
CONTE, M., GODEAU, P.: Cancer du pancréas et diabète. Presse méd. **68**, 2331 (1960).
CUTFORTH, R.H., POWELL, M.E.A.: Diabetes mellitus caused by secondary cancer of the pancreas. Brit. med. J. **1958 I**, 205.
ELLINGER, F., LANSMAN, H.: Frequency and course of cancer in diabetics. N. Y. St. J. Med. **44**, 259 (1944).
GLICKSMAN, A.S., RAWSON, R.W.: Diabetes and altered carbohydrate metabolism in patients with cancer. Cancer (Philad.) **9**, 1127 (1956).
GREEN, R.C., BAGGENSTOSS, A.H., SPRAGUE, R.G.: Diabetes mellitus in association with primary carcinoma of the pancreas. Diabetes **7**, 308 (1958).
KEEN, H.: Metastasizing carcinoid with diabetes mellitus. Diabetes **8**, 392 (1959).
MARBLE, A.: Cancer and diabetes. In: JOSLIN et al., Treatment of Diabetes mellitus, 10. Aufl., S. 577. Philadelphia: Lea & Febiger 1959.
MEAD, J.A., SMITH, B.M.: Diabetic acidosis with pancreatic artery thrombosis: unusual complication of carcinoma of the pancreas. J. Amer. Geriat. Soc. **11**, 369 (1963).
MILLER, M.: Cancer and diabetes. In: WILLIAMS, Diabetes, p. 719. New York: Paul B. Hoeber 1960.
SAYLE, B.A., LANG, A., GREEN, W.O., BOSWORTH, W.C., GREGORY, R.: Cushing syndrome due to carcinoma of the pancreas. Ann. intern. Med. **63**, 58 (1965).
VANDER, J.B.: The association of diabetes mellitus and carcinoma of the endometrium. Amer. J. Obstet. Gynec. **77**, 243 (1959).
WARREN, S., LECOMPTE, P.: Pathology of diabetes mellitus, 3. Aufl. Philadelphia: Lea & Febiger 1952.

Pregnancy

BORONOW, R.C., MCELIN, TH.W.: Diabetes in pregnancy. Amer. J. Obstet. Gynec. **91**, 1022 (1965).
BURT, R.L.: Glucose tolerance in pregnancy. Diabetes **11**, 227 (1962).
CONSTAM, G.R.: Diabetes mellitus. In: MUELLER u. STUCKI, Richtlinien zur medizinischen Indikation der Schwangerschaftsunterbrechung, S. 86. Berlin-Göttingen-Heidelberg-New York: Springer 1964.
– Einfluß der Schwangerschaft auf den Diabetes mellitus. Bull. schweiz. Akad. med. Wiss. **21**, 77 (1965).
— Diabetes und Schwangerschaft. Intern-medizinische Probleme. Gynaecologia (Basel) **159**, 193 (1965).
– Der Diabetes während der Schwangerschaft. Praxis **55**, 1356 (1966).
– JOST, L., RUST, TH., WILLI, H.: Diabetes und Schwangerschaft. Schweiz. med. Wschr. **93**, 1611 (1963).
– RUST, TH., WILLI, H.: Resultate langjähriger Erfahrung. Bull. schweiz. Akad. med. Wiss. **21**, 117 (1965).
— — — Diabetes and Pregnancy. Acta diabet. lat. **8**, 26 (1971).
CORNBLATH, M., REISNER, H.: Blood glucose in the neonate and its clinical significance. New Engl. J. Med. **273**, 387 (1965).
FARQUHAR, J.W., ISLES, T.E.: Glycémies et comportement clinique chez les nouveau-nés de mères normales et diabétiques. Journées Ann. Diabét. Hôtel Dieu Paris **8**, 99 (1967).
FLYNN, F.V., HARPER, C., MAYO, P. DE: Lactosuria and glycosuria in pregnancy and the puerperium. Lancet **1953 II**, 698.
GILBERT, J.A.L., DUNLAP, D.M.: Diabetic maternal mortality and fetal loss rate. Brit. med. J. **1949 I**, 48.
GLEISS, J., HUETER, K., JAHNKE, K., DAWEKE, H., SACHSSE, B.: Zum Gestaltwandel der Neugeborenen diabetischer Mütter. Dtsch. med. Wschr. **91**, 257 (1966).
GREENE, J.W., SMITH, K., KYL, G.C., TOUCHSTONE, J.C., DUHRING, J.L.: The use of urinary estriol excretion in the management of pregnancies complicated by diabetes mellitus. Amer. J. Obstet. Gynec. **91**, 684 (1965).
HAGBARD, L.: Pregnancy and diabetes mellitus. Springfield: Ch.C. Thomas 1961.
HILIS, G., VENNING, E.H., DOHAN, F.C., WEBSTER, G.D., RICHARDSON, E.M.: Pregnancy and adrenocortical function. J. clin. Invest. **33**, 1466 (1954).
HOET, J.P., HOET, J.J., GOMMERS, A.: Endocrine disturbances of pregnancy and foetal pathology. Proc. roy. Soc. Med. **52**, 813 (1956).
HORKY, Z., FISCHER, U., WAPPLER, E.: Schwangerschaft bei Diabetes mellitus. Stuttgart: Fischer 1969.
JOHN, H.L.: Prediabetics, what becomes of them. Amer. J. dig. Dis. **17**, 219 (1950).
JOST, L.: Beobachtungen an 100 Geburten diabetischer Frauen. Arch. Gynäk. **199**, 209 (1963).
KYLE, G.C.: Diabetes and pregnancy. Ann. intern. Med., Suppl. 3, **59** (1963).
– YALCIN, S., GREENE, J.W., JR., SMITH, K.: Urinary oestriol excretion in the pregnant diabetic. In: LEIBEL and WRENSHALL, On the nature and treatment of diabetes, p. 700. Amsterdam: Excerpta med. Found. 1965.
MALINS, J.M., FITZGERALD, M.G.: Childbearing prior to recognition of diabetes. Diabetes **14**, 175 (1965).
MILLER, H.C.: Offspring of diabetic and prediabetic mothers. Advanc. Pediat. **8**, 137 (1956).
MOHNIKE, G.: Schwangerschaft und Neugeborene der zukkerkranken Frau. Berlin: Volk und Gesundheit 1966.
OAKLEY, W.: The treatment of pregnancy in diabetes mellitus. In: LEIBEL and WRENSHALL, On the nature and treatment of diabetes, p. 673. Amsterdam: Excerpta med. Found. 1965.
OSLER, M.: Structural and chemical changes in infants of diabetic and prediabetic mothers. In: LEIBEL and WREN-

SHALL, On the nature and treatment of diabetes, p. 692. Amsterdam: Excerpta med. Found. 1965.
PEDERSEN, J.: Diabetes and pregnancy. Copenhagen: J. Pedersen 1954.
POMERANZE, J., STONE, L., KING, H.J.: The obstetric importance of obesity and "benign" glycosuria in prediagnosis diabetes. Obstet. and Gynec. **13**, 181 (1959).
RUST, TH.: Der Einfluß des Diabetes auf die Schwangerschaft. Bull. schweiz. Akad. med. Wiss. **21**, 90 (1965).
— Geburtshilfliche Aspekte. Gynaecologia (Basel) **159**, 215 (1965).
— Schwangerschaft bei zuckerkranken Frauen. Praxis **55**, 1361 (1966).
SIEGLER, H.-J.: Die Bedeutung der Schwangerschaftsfürsorge bei Diabetikerinnen. Med. Welt 265 (1961).
SIREK, O.V.: Serum protein in newborn infants of diabetic mothers. In: LEIBEL and WRENSHALL, On the nature and treatment of diabetes, p. 727. Amsterdam: Excerpta med. Found. 1965.
SPELLACY, W.N., CARLSON, K.L., BIRK, S.A.: Effect of posterior pituitary hormones on blood glucose and plasma insulin levels in postpartum patients. Obstet. and Gynec. **28**, 355 (1966).
— — — The fetus and the placenta a source of insulin during pregnancy. Obstet. and Gynec. **29**, 74 (1967).
— GOETZ, F.C., GREENBERG, F.Z., JOYCE, E.: Plasma insulin in normal midpregnancy. Amer. J. Obstet. Gynec. **92**, 11 (1965).
STEINKE, J., DRISCOLL, S.G.: The extractable insulin content of pancreas from fetuses and infants of diabetic and control mothers. Diabetes **14**, 573 (1965).
THOMAS, K., GASPARO, M.DE, HOET, J.J.: Insulin levels in the umbilical vein and in the umbilical artery of normal and gestational diabetic mothers. Diabetologia **3**, 299 (1967).
WHITE, P.: Pregnancy complicating diabetes, In: JOSLIN et al., Treatment of Diabetes mellitus, 10. Aufl. Philadelphia: Lea & Febiger 1959.
WILLI, H.: Einfluß des mütterlichen Diabetes auf das Kind. Bull. schweiz. Akad. med. Wiss. **21**, 110 (1965).
WILSON, J.S.P., VALLANCE-OWEN, J.: Congenital deformities and insulin antagonism. Lancet **1966II**, 940.
WORM, N.: Diabetes mellitus und Schwangerschaft. Berlin: Akademie-Verlag 1959.

Infantile and Juvenile Diabetes Mellitus

BERTOYE, P., STEFANI, A.: Le diabète chez le nourrisson. Pédiatrie **9**, 147 (1954).
BICKEL, H.: Die nicht-diabetischen Melliturien des Kindes. Med. Probl. Paediat. **6**, 313 (1960).
BIHRER, R.: Erkrankungsalter und Wachstumsverlauf beim jugendlichen Diabetes mellitus in der ärztlichen Praxis. Helv. paediat. Acta **25**, 312 (1970).
BLEGER, G.: Le développement mental et psycho-affectif et l'âge du jeune diabétique. Diabète (Le Raincy) **11**, 314 (1963).
BRODRIBB, H.S., MCMURRAY, J., SCOTT, L.G.: Two cases of diabetes mellitus in infants under 1 year. Brit. med. J. **1952I**, 1060.
ELLIOTT, R.B., O'BRIEN, D., ROY, C.C.: An abnormal insulin in juvenile diabetes mellitus. Diabetes **14**, 780 (1965).
FARRELL, H.W., HAND, A.M., NEWCOMB, A.L.: Infantile diabetes. Diabetes **2**, 88 (1953).
GANS, B.: Diabetes mellitus in an infant aged 39 days. Proc. roy. Soc. Med. **47**, 132 (1954).
GUENTHER, O.: Zur Größenentwicklung heranwachsender Diabetiker. 4. Kongr. Internat. diab. Fed. Genf Méd. and Hyg., 1961, p. 357.
HAGER, H., HERBST, R.: Das transitorische Diabetes-mellitus-Syndrom des Neugeborenen, ein Krankheitsbild sui generis. Z. Kinderheilk. **95**, 324 (1966).
JOHN, H.J.: Diabetes mellitus in children. J. Pediat. **35**, 723 (1949).
KOPECKY, A., KELLEN, J.: Papierchromatographie der Zukker in der Klinik. Leipzig: G. Thieme 1963.
LISTER, Y.: The clinical spectrum of juvenile diabetes. Lancet **1966I**, 386.
MAURIAC, P.: Hepatomégalie de l'enfance avec troubles de la croissance et du métabolisme des glucides. Paris méd. **2**, 525 (1934).
— La croissance de l'enfant diabétique et le syndrome de Mauriac. Scalpel (Brux.) **107**, 1255 (1954).
NACHTIGALL, CH.: Beitrag zum Diabetes mellitus im Säuglingsalter. Mschr. Kinderheilk. **12**, 313 (1964).
OSEID, S., AAGENAES, O.: Insulin studies in a patient with temporary idiopathic neonatal hyperglycemia. Diabetologia **4**, 312 (1968).
PERELMAN, R., LESTRADET, H.: Diabète néonatal. Ann. Pédiat. **40**, 283 (1964).
ROHRACHER, T.: Spätschicksale zuckerkranker Kinder. In: Wiener Beiträge zur Kinderheilkunde, Bd. 3. Wien: Wilh. Maudrich 1951.
ROYER, P., LESTRADET, H.: Traitement du diabète infantile en régime libre. Paris: Flammarion 1958.
SCHWARTZMAN, J., CRUSIUS, M.E., BEIRNE, D.P.: Diabetes mellitus in infants under one year of age. Amer. J. Dis. Child. **74**, 587 (1947).
STRUWE, FR.E.: Zur Manifestation des Diabetes mellitus im Kindesalter. Mschr. Kinderheilk. **108**, 487 (1960).
TRAISMAN, H.S., NEWCOMB, H.L.: Management of juvenile diabetes. St. Louis: C.V. Mosby 1965.
UHRY, P., DUCAS, P.: Le diabète infantile et juvénile. Paris: Masson & Cie. 1955.
WEIL, W.B., ACK, M.: School achievement in juvenile diabetes mellitus. Diabetes **13**, 303 (1964).
WEISSE, K.: Behandlung diabetischer Kinder. Stuttgart: G. Thieme 1951.
WILLI, H., KOLLER, TH.: Mutter und Kinder, 23. Aufl. Zürich: Schulthess & Co. 1963.
— MÜLLER, F.: Über den transitorischen Diabetes mellitus des Neugeborenen. Helv. paediat. Acta **23**, 231 (1968).
WYLIE, E.S.: A case of congenital diabetes. Arch. Dis. Childh. **28**, 297 (1953).

Surgical Procedures in Diabetes Mellitus

COLE, W.H., SADOVE, M.: The need for complete cooperation between surgeon and anesthesiologist. J. Amer. med. Ass. **162**, 437 (1956).
CONSTAM, G.R.: Die Beurteilung der Operabilität von Zukkerkranken, ihre prae- und postoperative Behandlung. Schweiz. med. Wschr. **72**, 1289 (1942).
DRUCKER, W.R., COSTLEY, CH., STULTS, R., GROSS, G., HOLDEN, W., MILLER, M., CRAIG, J., WOODWARD, H.: The effect of anesthesia on intermediary carbohydrate metabolism. Clin. Res. Proc. **3**, 202 (1955).
DUNCAN, G.G., JENSON, W.K.: The surgical patient with diabetes. Amer. J. Cardiol. **12**, 636 (1963).
FORSHAM, P.: Management of diabetes during stress and surgery. In: WILLIAMS, Diabetes, p. 514. New York: P.B. Hoeber 1960.
GRIFFITH, J.A.: The effects of general anaesthesia and hexamethonium on the blood sugar in nondiabetic and diabetic surgical patients. Quart. J. Med. **22**, 405 (1953).
LAWSON, P.D., GREIG, M.E., ROBBINS, B.H.: The potentiating effect of glucose and its metabolic products on barbiturate anesthesia. Science **110**, 690 (1949).
MARBLE, A., STEINKE, J.: Physiology and pharmacology in diabetes mellitus. Anesthesiology **24**, 442 (1963).
MCKITTRICK, J.B.: Surgical procedures. In: DANOWSKI, Diabetes mellitus, diagnosis and treatment, p. 145. New York: Amer. Diab. Assn. 1964.
MONCRIEF, J.A., COLDWATER, K.B., ELMAN, R.: Postoperative loss of sugar in urine following intravenous infusion of fructose. Arch. Surg. **67**, 57 (1953).

Diabetes Mellitus after Removal or Destruction of the Pancreas

CREUTZFELDT, W., KUEMMERLE, F., KERN, E.: Beobachtungen an 4 Patienten mit totaler Duodeno-Pankreatektomie wegen eines Carcinoms des Pankreas. Dtsch. med. Wschr. **84**, 541 (1959).

DIXON, C.F., COMFORT, M.W., LICHTMAN, A.L., BENSON, R.E.: Total pancreatectomy for carcinoma of the pancreas in a diabetic person. Arch. Surg. **52**, 619 (1946).

DOYLE, A.P., BALCERZAK, S.P., JEFFREY, W.L.: Fatal diabetic glomerulosclerosis after total pancreatectomy. New Engl. J. Med. **270**, 623 (1964).

HARTER, M.M., KUHL, W.J.: The effect of pancreatectomy on a patient with preexisting diabetes mellitus. Diabetes **11**, 118 (1962).

KOCH, E., SAILER, F.X.: Mehrjährige Beobachtungen an zwei Totalpancreatectomierten. Dtsch. med. Wschr. **88**, 2499 (1963).

MILLER, M.: Diabetes associated with acromegaly, hyperadrenocorticism, hemochromatosis, pancreatitis, pancreatectomy and cancer. In: WILLIAMS, Diabetes, p. 719. New York: P.B. Hoeber 1960.

SALEMBIER, Y.: Un cas de pancréatectomie totale. Arch. Mal. Appar. Dig. **52**, 712 (1963).

WHITFIELD, A.G.W., CRANE, C.W., FRENCH, J.M., BALEY, T.J.: Life without pancreas. Lancet **1965I**, 675.

Hemochromatosis

ALLAN, F.H., CONSTAM, G.R.: Insulin resistance in a case of bronze diabetes. Med. Clin. N. Amer. **12**, 1677 (1929).

DAVIS, N.O., ARROWSMITH, W.R.: The treatment of hemochromatosis by massive venesection. Ann. intern. Med. **39**, 723 (1953).

FRANCON, F., EPINEY, J., BLANCHARD, H., JOLY, L., VISNIKS, E., DIAZ, R.: Contribution à l'étude des arthropathies de l'hemochromatose. Presse méd. **76**, 1809 (1968).

HEDINGER, CH.: Zur Pathologie der Hämochromatose. Hämochromatose als Syndrom. Helv. med. Acta, Suppl. 32, zu **20** (1953).

WOEHLER, F.: Therapie der Hämochromatose. Dtsch. med. Wschr. **90**, 348 (1965).

Mucoviscidosis

ANDERSON, J.W., SAWYER, K.C., SHERIDAN, P.: Cystic fibrosis of the pancreas with diabetes mellitus. Rocky Mnt med. J. **60**, 31 (1963).

CHARLES, R.N., KELLEY, M.L.: Occurrence of diabetes mellitus in families of patients with cystic fibrosis of the pancreas. J. chron. Dis. **14**, 381 (1961).

DI S. AGNESE, P.A., TALAMO, R.C.: Cystic fibrosis of the pancreas. New Engl. J. Med. **277**, 1287 (1967).

FRANCOIS, R., MANTANUS, R.F., Mme RUITON-UGLIENGO: Etude de test de la sueur chez les enfants diabétiques. Diabète (Le Raincy) **11**, 344 (1963).

ISRAEL, L., CHIMÉNES, H., DEBRIS, M., KLOTZ, H.P.: Incidence et role étiologique de la mucoviscidose dans le diabète de l'adulte. Presse méd. **69**, 176 (1961).

KOCH, E., LEHMANN, W., RICK, W., GUMBEL, W.: Mucoviscidosis-Symptome beim Diabetes mellitus. Dtsch. med. Wschr. **86**, 1433 (1961).

LOWE, CH.U., ADLER, W., BROBERGER, O., WALSH, J., NETTER, E.: Mucopolysaccharides from patients with cystic fibrosis of pancreas. Science **153**, 1124 (1966).

PROTO, R., NARDELLI, G., GIORGINO, R.: Mucoviscidosi latente in diabetici adulti. Acta diabet. lat. **2**, 350 (1965).

STEINBERG, A.G., BROWN, D.C.: On the incidence of cystic fibrosis of the pancreas. Amer. J. Genet. **12**, 416 (1960).

WILROY, R.S., CRAWFORD, S.E., JOHNSON, W.W.: Cystic fibrosis with extensive fat replacement of the liver. J. Pediat. **68**, 67 (1966).

Special Forms of Diabetes Mellitus

CAMPBELL, G.D.: Insulin-independent young diabetics in Natal. Brit. med. J. **1960II**, 537.

CRAIG, J.W., MILLER, M.: Lipoatrophic diabetes. 4. Kongr. Internat. Diab. Fed. Genf Méd. and Hyg., 1961, p. 162.

DEROT, M., ROSSELIN, G., ASSAN, R., TCHOBROUTSKY, G.: Taux plasmatiques de l'insuline et de l'hormone de croissance dans deux cas de diabète lipoatrophique. Diabetologia **2**, 147 (1966).

DRAPKIN, A.: Lipoatrophic diabetes. In: ELLENBERG and RIFKIN, Clinical Diabetes mellitus, p. 417. New York: McGraw-Hill Book Co. 1962.

HAMWI, G.J., KRUGER, F.A., EYMOTT, M.J., SCARPELLI, D.G., GWINUP, G., BYRON, R.: Lipoatrophic diabetes. Diabetes **15**, 262 (1966).

HUGH-JONES, P.: Diabetes in Jamaica. Lancet **1955II**, 891.

LABHART, A., PRADER, A., GINSBERG, J.: Der myatonische Diabetes. In: PFEIFFER, E., Handbuch des Diabetes mellitus. München: J.F. Lehmann 1968.

LAWRENCE, R.D.: Types of human diabetes. Brit. med. J. **1951I**, 373.

LOUIS, L.H., CONN, J.W., MINICK, N.C.: A diabetogenic polypeptide from bovine adenohypophysis similar to that excreted in lipoatrophic diabetes. Metabolism **15**, 309 (1966).

MARCUS, R.: Retinopathy, nephropathy and neuropathy in lipoatrophic diabetes. Diabetes **15**, 351 (1966).

PAVEL, I., CIMPEANU, S., NICOLESCO, M., BONAPARTE, H., PETROVICI, G., STOLAN, N.: Le diabète lipo-atrophique est-il un diabète lipidique? Presse méd. **71**, 1279 (1963).

PRADER, A., LABHART, A., WILLI, H.: Ein Syndrom von Adipositas, Kryptorchismus und Oligophrenie nach myatonieartigem Zustand im Neugeborenenalter. Schweiz. med. Wschr. **86**, 1269 (1956).

— WILLI, H.: Das Syndrom von Imbezillität, Adipositas, Muskelhypotonie, Hypogenitalismus, Hypogonadismus und Diabetes mellitus mit „Myatonie"-Anamnese. Verhdlg. 2. intern. Kongr. psych. Entw. Stör. Kindesalter, Wien 1961, Pat. I, S. 353 (1963).

ROYER, P.: Le diabète sucré dans le syndrome de Willi-Prader. Journées Ann. Diabét. Hôtel Dieu Paris **4**, 91 (1963).

SALTIEL, H.: Le diabète lipoatrophique à la lumière d'une trentaine de cas connus. I-Diss. Paris, 1964.

SCHWARTZ, R., SCHAFER, A., RENOLD, A.E.: Generalized lipoatrophy, hepatic cirrhosis, disturbed carbohydrate metabolism and accelerated growth. Amer. J. Med. **28**, 973 (1960).

SHAPERA, G.: The pattern of diabetes in Africans in Uganda. In: OBERDISSE u. JAHNKE, Diabetes mellitus, S. 664. Stuttgart: G. Thieme 1959.

TRIPATHY, B.B., KAR, B.C.: Observations on clinical patterns of diabetes mellitus in India. Diabetes **14**, 404 (1965).

TULLOCH, J.A.: Diabetes mellitus in the tropics. London: E. & S. Livingstone 1962.

— MCINTOSH, D.: "J"-type diabetes. Lancet **1961II**, 119.

ZOYSA, V.P. DE: Clinical variations of the diabetic syndrome in a tropical country (Ceylon). Arch. intern. Med. **88**, 818 (1951).

Diagnosis of Diabetes Mellitus

Reviews

1. FREHNER, H.U.: Diabetes-Fibel, 3. Auflage. Stuttgart: Geoerg Thieme 1972.
2. KNICK, B.: Diabetes-Diagnostik. Studienreihe Boehringer-Mannheim, 1967.
3. MALINS, J.: Clinical Diabetes mellitus. London: Eyre & Spottiswoode 1963.
4. OAKLEY, W.G., PYKE, D.A., TAYLOR, K.W.: Clinical diabetes and its biochemical basis. Oxford and Edinburgh: Blackwell Scientific Publications 1968.

AMATUZIO, D.S., STUTZMAN, F.L., VANDERBILT, M.J., NESBIT, S.: Interpretation of the rapid intravenous glucose tolerance test in normal individuals and in mild diabetes mellitus. J. clin. Invest. **32**, 428 (1953).
BERSON, S.A., YALOW, R.S.: Immunoassay of plasma insulin. In: Ciba Foundation Coll. on Endocrinology **14**, 182 (1962).
CONRAD, V.: Mesure de l'assimilation de Glucose: Bases théoriques et applications cliniques. Acta gastro-ent. belg. **18**, 655, 727, 803 (1955).
ELLENBERG, M., RIFKIN, H.: Diabetes mellitus: Theory and practice. New York: McGraw-Hill Book Company 1970.
FAJANS, S.S.: Cortisone glucose tolerance test. J. Amer. med. Ass. **186**, 279 (1963).
FINE, J.: Glucose content of normal urine. Brit. med. J. **1965 I**, 1209.
FREHNER, H.U.: Diabetes-Fibel für Ärzte und Studierende, 3. Aufl. Stuttgart: Thieme 1972.
FROESCH, E.R., RENOLD, A.E.: Specific enzymatic determination of glucose in blood and urine using glucose oxidase. Diabetes **5**, 1 (1956).
HALES, C.N., RANDLE, P.J.: Immunoassay of insulin with insulin antibody precipitate. Biochem. J. **88**, 137 (1963).
HAYNER, N.S., KJELSBERG, M.O., EPSTEIN, F.H., FRANCIS, T., JR.: Carbohydrate tolerance and diabetes in a total community, Tecumseh, Michigan. I. Effects of age, sex, and test conditions on one-hour glucose tolerance in adults. Diabetes **14**, 413 (1965).
JARRETT, R.J., KEEN, H.: Further observations on the diurnal variation in oral glucose tolerance. Brit. med. J. **1970 IV**, 334.
LONG, C., KILO, C., RECANT, L.: Experience with a steroid-tolbutamide test for the detection of impaired pancreatic reserve. Diabetes **13**, 127 (1964).
MCDONALD, G.W., FISHER, G.F., BURNHAM, C.: Reproducibility of the oral glucose tolerance test. Diabetes **14**, 473 (1965).
ROST, G., HONIGMANN, G., SCHLIACK, V.: Altersabhängige Einordnungskriterien der oralen 50 g-Glukose-Belastung zur Labordiagnostik des Diabetes mellitus. Z. Ges. inn. Med. **22**, 709 (1967).
TEUSCHER, A., RICHTERICH, R., BURGI, W., DETTWILER, W., ZUPPINGER, K.: Neue schweizerische Richtlinien zur Diagnose des Diabetes mellitus. Schweiz. med. Wschr. **101**, 345, 390 (1971).
UNGER, R.H., MADISON, L.L.: Intravenous tolbutamide test. In: DANOWSKI, T.S. (ed.), Diabetes mellitus. Diagnosis and treatment, p. 41. New York 1964.
U. S. Dept. of Health, Education and Welfare: Response of 24 middle-aged men to 50 and 100 g doses of glucose given fasting and 1, 2, and 3 hours after meal. National Center for Health Statistics, Ser. 11, No 2, 1963.
WILLIAMS, R.: The chromatographic separation of reducing sugar in urine. J. med. Lab. Technol. **12**, 43 (1954).

Pathophysiology and Clinical Features of Hypoglycemia

Reviews

BOSHELL, B.R.: Treatment of hypoglycemia. Mod. Treatm. **3**, 329 (1966).
CONN, J.W., SELTZER, H.S.: Spontaneous hypoglycemia. Amer. J. Med. **19**, 460 (1955).
CORNBLATH, M.: Hypoglycemia. In: Carbohydrate metabolism and its disorders, vol. 2 (DICKENS, H., RANDLE, R.J., and WHELAN, W.J., eds.), p. 51. London and New York: Academic Press 1968.
MCQUARRIE, I.: Idiopathic spontaneously occurring hypoglycemia in infants. Amer. J. Dis. Child. **87**, 399 (1954).
MARKS, V., ROSE, F.C.: Hypoglycemia. Oxford: Blackwell Scientific Publications 1965.
OBERDISSE, K. (Hrsg.): Tagung der Deutschen Endokrinologen-Gesellschaft. Berlin-Heidelberg-New York: Springer 1968.
SKILLERN, P.G., RYNEARSEN, E.H.: Medical aspects of hypoglycemia. J. clin. Endocr. **13**, 587 (1953).
STANBURY, J.B., WYNGAARDEN, J.B., FREDRICKSON, D.S. (Hrsg.): The metabolic basis of inherited disease. Third edition. New York: McGraw-Hill Book Co. 1972.

Main Forms of Hypoglycemia

ARKY, R.A., FREINKEL, N.: Alcohol hypoglycemia. V. Alcohol infusion to test gluconeogenesis in starvation, with special reference to obesity. New Engl. J. Med. **274**, 426 (1966).
BAGDADE, J.D., BIERMAN, E.L., PORTE, D., JR.: The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J. clin. Invest. **46**, 1549 (1967).
BAKER, L., WINEGRAD, A.I.: Fasting hypoglycemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphate activity. Lancet **1970 II**, 13.
BROBERGER, O., ZETTERSTRÖM, R.: Hypoglycemia with an inability to increase the epinephrine secretion in insulin-induced hypoglycemia. J. Pediat. **59**, 215 (1961).
COCHRANE, W.A., PAYNE, W.W., SIMPKISS, M.J., WOOLF, L.I.: Familial hypoglycemia precipitated by amino acids. J. clin. Invest. **35**, 411 (1956).
CORREDOR, C., BRENDEL, K., BRESSLER, R.: Studies on the mechanism of the hypoglycemic action of 4-Pentenoic acid. Proc. nat. Acad. Sci. (Wash.) **58**, 2299 (1967).
EULER, U.S. VON, IKKOS, D., LUFT, R.: Adrenaline excretion during resting conditions and after insulin in adrenalectomized human subjects. Acta endocr. (Kbh.) **38**, 441 (1961).
FAJANS, S.S., FLOYD, J.C., JR., KNOPF, R.F., RULL, J., GÜNTSCHE, E.M., CONN, J.W.: Benzothiadiazine suppression of insulin release from normal and abnormal islet tissue in man. J. clin. Invest. **45**, 481 (1966).
— SCHNEIDER, J.M., SCHTEINGART, D.E., CONN, J.W.: The diagnostic value of sodium tolbutamide in hypoglycemic states. J. clin. Endocr. **21**, 371 (1961).
FIELD, R.A.: Glycogen deposition diseases. In: STANBURY, J.B., WYNGAARDEN, J.B., and FREDRICKSON, D.S. (Hrsg.), The metabolic basis of inherited disease, p. 141. New York: McGraw-Hill Book Co. 1966.
FLOYD, J.C., JR., FAJANS, S.S., CONN, J.W., KNOPF, R.F., RULL, J.: Stimulation of insulin secretion by amino acids. J. clin. Invest. **45**, 1487 (1966).
— — KNOPF, R.F., CONN, J.W.: Plasma insulin in organic hyperinsulinism: comparative effects of tolbutamide, leucine and glucose. J. clin. Endocr. **24**, 747 (1964).
FREINKEL, N., COHEN, A.K., ARKY, R.A., FOSTER, A.E.: Alcohol hypoglycemia: II. A postulated mechanism of action based on experiments with rat liver slices. J. clin. Endocr. **25**, 76 (1965).
FROESCH, E.R.: Essential fructosuria and hereditary fructose intolerance. In: STANBURY, J.B., WYNGAARDEN, J.B., and FREDRICKSON, D.S. (Hrsg.), The metabolic basis of inherited disease, p. 124. New York: McGraw-Hill Book Co. 1966.
— BÜRGI, H., ZIEGLER, W., BALLY, P., LABHART, A.: Zur Pathogenese der tumorbedingten Hypoglykämie ohne Hyperinsulinismus. Schweiz. med. Wschr. **36**, 1250 (1963).
— JAKOB, A., LABHART, A.: Hypoglykämie bei extrapankreatischen Tumoren. In: Symposium der Deutschen Gesellschaft für Endokrinologie, 1968, im Druck.
ISSELBACHER, K.J.: Galactosemia. In: STANBURY, J.B., WYNGAARDEN, J.B., and FREDRICKSON, D.S. (Hrsg.), The metabolic basis of inherited disease, p. 178. New York: McGraw-Hill Book Co. 1966.
JAKOB, A., MEYER, U.A., FLURY, R., ZIEGLER, W.H., LABHART, A., FROESCH, E.R.: The pathogenesis of tumour

hypoglycaemia: Blocks of hepatic glucose release and of adipose tissue lipolysis. Diabetologia **3**, 506 (1967).
JELIFFE, D.B., STUART, K.: Acute toxic hypoglycaemia in the vomiting sickness of Jamaica. Brit. med. J. **1954I**, 75.
LUNDBAEK, K., LYNGSØE, J., MADSEN, B., YDE, H., ØRSKOV, H.: The diagnosis of insuloma. Acta med. scand. **181**, 269 (1967).
MCQUARRIE, I.: Idiopathic spontaneously occuring hypoglycemia in infants. Amer. J. Dis. Child. **87**, 399 (1954).
MARKS, V., MARRACK, D.: Glucose assimilation in hyperinsulinism. A critical evaluation of the intravenous glucose tolerance test. Clin. Sci. **23**, 103 (1962).
— ROSE, F.C.: Hypoglycemia, p. 136—154. Oxford: Blackwell Scientific Publications 1965.
— SAMOLS, E.: Diagnostic tests for evaluating hypoglycemia. Proc. of the 6th Internat. Diabetes Congress, in press.
MARKS, V., MARRACK, D.: The glucagon test for insulinoma; a chemical study in 25 cases. J. clin. Path., in press.
MOSS, N.H., RHOADES, J.E.: Hyperinsulinism and islet cell tumors of the pancreas. In: Surgical diseases of the pancreas (HOWARD, J.M., and JORDAN, G.L., eds.), p. 321. London: Pitman 1960.
PAPAIOANNOU, A.N.: Tumors other than insulinomas associated with hypoglycemia. Surg. Gynec. Obstet. **123**, 1093 (1966).
PERLEY, M.J., KIPNIS, D.M.: Plasma insulin responses to oral and intravenous glucose: Studies in normal and diabetic subjects. J. clin. Invest. **46**, 1954 (1967).
RAY, P.D., FOSTER, D.O., LARDY, H.A.: Paths of carbon in gluconeogenesis. IV. Inhibition by 1-tryptophan of hepatic gluconeogenesis at the level of phosphoenolpyruvate formation. J. biol. Chem. **241**, 3904 (1966).
SAMOLS, E., MARKS, V.: Insulin assay in insulinomas. Brit. med. J. **1963I**, 507.
SELTZER, H.S., FAJANS, S.S., CONN, J.W.: Spontaneous hypoglycemia as an early manifestation of diabetes mellitus. Diabetes **5**, 437 (1956).
SILVERSTEIN, M.N., WAKIM, K.G., BAHN, R.C.: Further observations on the role of tryptophan and its metabolites in hypoglycemia associated with neoplasia. Clin. Res. Proc. **13**, 334 (1965).
SMITH, W.H., FRASER, R., STAYNES, K., WILLCOX, J.M.: The causes of postprandial attacks of palpitation and weakness after gastric operations. Quart. J. Med. **22**, 381 (1953).
SOKAL, J.R.: Glucagon — an essential hormone. Amer. J. Med. **41**, 331 (1966).
STALKER, M., LEWIS, R.C., SUTHERLAND, W.H., BOGOCH, A.: Carbohydrate metabolism in patients after partial gastrectomy. Canad. med. Ass. J. **80**, 779 (1955).
STEINKE, J.: Pathophysiologie und Klinik des organischen Hyperinsulinismus. In: Verhandl. des Symposiums der Deutschen Gesellschaft f. Endokrinologie, 1968, im Druck.
UNGER, R.H.: The riddle of tumor hypoglycemia. Amer. J. Med. **40**, 325 (1966).
YALOW, R.S., BERSON, S.A.: Immunoassay of endogenous plasma insulin in man. J. clin. Invest. **39**, 1157 (1960).

Glucagon

Reviews and Books

FOA, P.P.: Glucagon. In: Ergebnisse der Physiologie, biologischen Chemie und experimentellen Pharmakologie, Bd. 60, S. 141. Berlin-Heidelberg-New York: Springer 1968.
KRACHT, J.: Nebenschilddrüse und endokrine Regulationen des Calciumstoffwechsels. Spontanhypoglykämie. Glukagon. 14. Symp. Dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.
LEFEBVRE, P.: Le glucagon, seconde hormone pancréatique. Bruxelles: Editions Arscia S.A. 1967.
WEINGES, K.F.: Glucagon. In: G. WEITZEL und N. ZÖLLNER (Hrsg.), Biochemie und Klinik. Stuttgart: Georg Thieme 1968.

Chemistry, Sources and Metabolic Effects

BENCOSME, S.A., FREI, J.: Relation of glucagon to A-cells of the pancreas. Proc. Soc. exp. Biol. (N.Y.) **91**, 589 (1956).
BIRGE, S.J., AVIOLI, L.V.: Glukagon-induced hypocalcemia in man. J. clin. Endocr. **29**, 213 (1969).
BROMER, W.W., SINN, L.G., STAUB, A., BEHRENS, O.K.: The amino acid sequence of glucagon. J. Amer. chem. Soc. **78**, 3858 (1956).
GIBBS, C.B.F., ROOT, E.W., JR., MURLIN, J.R.: The glucogenic substance in extracts of pancreas and other tissues. Quart. J. exp. Physiol. **13** (Suppl.), 128 (1923).
KIMBALL, C.P., MURLIN, J.R.: Aqueous extracts of pancreas. III. Some precipitation reactions of insulin. J. biol. Chem. **58**, 337 (1923).
KRACHT, J.: Nebenschilddrüse und endokrine Regulationen des Calciumstoffwechsels. Spontanhypoglykämie. Glukagon. 14. Symp. Dtsch. Ges. Endokr. Berlin-Heidelberg-New York: Springer 1968.
MCLEOD, J.J.R.: The source of insulin: a study of the effect produced on blood sugar by extracts of the pancreas and principal islets of fishes. J. metab. Res. **2**, 149 (1922).
MURLIN, J.R., CLOUGH, H.D., GIBBS, C.B.F., STOKES, A.M.: Aqueous extracts of the pancreas. I. Influence on the carbohydrate metabolism of depancreatized animals. J. biol. Chem. **56**, 253 (1923).
SAMOLS, E., MARRI, G., MARKS, V.: Promotion of insulin secretion by glucagon. Lancet **1965II**, 415.
— — — Stimulation of glucagon secretion by oral glucose. Lancet **1965II**, 1257.
— TYLER, J., MEGYESI, C., MARKS, V.: Immunochemical glucagon in human pancreas, gut and plasma. Lancet **1966II**, 727.
SOKAL, J.E.: Effect of glucagon on gluconeogenesis by the isolated perfused rat liver. Endocrinology **78**, 538 (1966).
STAUB, A., SINN, L., BEHRENS, O.K.: Purification and crystallization of hyperglycemic-glycogenolytic factor (HGF). Science **117**, 628 (1953).
SUTHERLAND, E.W., CORI, C.F.: Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase. J. biol. Chem. **188**, 531 (1951).
— ØYE, I., BUTCHER, R.W.: The action of epinephrine and the role of the adenyl cyclase system in hormone action. Recent Progr. Hormone Res. **21**, 623 (1965).
UNGER, R.H., OHNEDA, A., VALVERDE, I., EISENTANT, A.M., EXTON, J.: Characterization of the response of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. J. clin. Invest. **47**, 48 (1968).
VECCHIO, D., LUYCKX, A., ZAHND, G.R., RENOLD, A.E.: Insulin release induced by glucagon in organ cultures of fetal rat pancreas. Metabolism **15**, 577 (1966).

Added in Proof:

BRODER, L.E., CARTER, S.K.: Pancreatic islet cell carcinoma. I. Clinical features of 52 patients. II. Results of therapy with streptozotocin. Ann. int. Med. **79**, 101 (1973).
ZINGG, A.E., FROESCH, E.R.: Effects of partially purified preparations with nonsuppressible insulin-like activity (NSILA-S) on sulfate incorporation into rat and chicken cartilage. Diabetologia **9**, 472 (1973).

XIV. The Parathyroids

M. WERNLY

With Contributions by

B. COURVOISIER and J. A. FISCHER

A. Historical Dates

1880 The parathyroids were described for the first time in man by SANDSTRÖM.
1895 KOHN added to SANDSTRÖM's description and recognized for the first time that the parathyroids were independent of the thyroid gland.
1896 VASSALE and GENERALI showed that removal of all four parathyroids produced severe tetany in dog.
1903 ASKANAZY found an adenoma of the parathyroid gland at the post mortem of a case of skeletal disease and suspected a connection between the two. This is the first reported case of hyperparathyroidism.
1906 ERDHEIM suspected the connection between the parathyroids and mineral metabolism.
1907 ERDHEIM described hyperplasia of several or all parathyroids in osteomalacia in man.
1908 MCCALLUM and VÖGTLIN successfully demonstrated that hypocalcemia arose after parathyroidectomy.
1914 ERDHEIM described hyperplasia of several or all the parathyroids in spontaneous rickets in rat. This is the earliest report of compensatory hyperplasia of the parathyroids, i.e. secondary hyperparathyroidism.
1926 MANDL successfully cured a clinical case of primary hyperparathyroidism by removing an adenoma of the parathyroid.
1925 COLLIP and HANSON, working independently, discovered parathyroid hormone and its hypercalcemic effect.
1934–1948 ALBRIGHT (Boston) described the biochemistry and diagnosis of primary hyperparathyroidism. He discovered that primary hyperparathyroidism was frequently an endocrine disorder, with nephrolithiasis as the most important symptom.
1959 RASMUSSEN and CRAIG and AURBACH succeeded in isolating and purifying parathyroid hormone and it was recognized as a polypeptide hormone.
1962 COPP isolated a hormonal factor with a hypocalcemic action from the parathyroids.
1963 HIRSCH showed that this hormone originated in the thyroid in mammals.
1963 BERSON described a radioimmunological method for the estimation of parathyroid hormone in serum, with which he was able to measure elevated values.
1965 TENENHOUSE showed that the hypocalcemic factor was also a polypeptide hormone. In birds and reptiles it is found in the ultimobranchial body. It is now generally called calcitonin rather than thyrocalcitonin.
1967 RAISZ succeeded in estimating the calcitonin concentration in plasma in several patients by recording the decrease in the number of osteoclasts in bone cultures *in vitro*.
Immunological methods introduced by TASHJIAN, DEFTOS, and ARNAUD have simplified the estimation of calcitonin.

B. Normal Anatomy and Histology

M. WERNLY

Weight. The 4 parathyroids in the unfixed state have a combined weight of 170 mg on average. The superior glands are somewhat smaller and each weighs 40 mg, as against the inferior glands, each of which weighs 45 mg (WERNLY, 1946).

Shape. The normal parathyroid is almost the size and shape of a lentil. It is flattened on both sides and is usually longitudinally oval. It is brownish red in color and has a yellow tint in the presence of an increased fatty penetration. The consistency is softer than that of a lymph node of the same size, and more compact than that of a fragment of fat.

Number. Normally, 4 parathyroid glands are formed in man. However, there have been reports of 2 or 3 glands. The possibility of congenital hypoplasia or aplasia cannot be excluded, but it is more likely that inadequate anatomical

preparation is the reason. We have demonstrated and histologically verified more than 4 glands in 50 cases among 450 post mortems. There are a few reports of even 8–12 glands (ERDHEIM and WERNLY, 1946).

Ontogenesis. The upper parathyroids arise from the 4th pharyngeal pouch and the lower parathyroids from the 3rd. The two pairs of glands cross each other during the developmental migration. The lower parathyroids move an especially long way, together with the thymus which also arises from the 3rd pharyngeal pouch. Occasionally their progress is arrested during this process or they descend further than usual. The numerous variations in the position of the lower parathyroids are thus due to the development.

Normal Topography. The superior parathyroids are usually situated on the posterior surface of the thyroid gland. They are generally connected with one of the hilus-like depressions in the middle of the two lobes of the thyroid gland, which is entered by a branch of the inferior thyroid artery. The lower parathyroids are normally embedded in a pyramid-shaped piece of fat at the lower pole of the thyroid gland. The base of this pyramid faces upwards and is formed by the thyroid gland. The two lateral surfaces are formed by the carotid artery and the trachea, and the anterior and posterior surfaces by the superficial and deep fasciae of the neck.

Arterial Blood Supply. Each parathyroid has its own artery, the parathyroid artery, which enters at the hilus and passes into the center of the gland from where it supplies the entire parenchyma via several branches. There are no anastomoses connecting the parenchyma with the surrounding tissues. The parathyroid artery is thus an end artery. Generally all four parathyroid arteries arise in the inferior thyroid artery, but occasionally the upper pair arise in an anastomosis between the inferior and superior thyroid arteries. There is a further anastomosis between the upper and lower thyroid arteries and the arteries of the pharynx, the esophagus, and the trachea, so that an adequate blood supply to the parathyroids is ensured in all circumstances. During radical neck surgery, and less frequently during operations on the thyroid, one or more of the parathyroids may be inadvertently removed or their blood supply endangered. Ligation of the small arterial branches in the region of the surface of the thyroid gland is thought to be especially dangerous. The branch which gives rise to the parathyroid artery can also be involved in this process. Ligation of this branch proximally instead of distally to the origin of the parathyroid artery, may cause necrosis of the parathyroid gland. HALSTED has therefore advocated that the small arterial branches be ligated near to the surface of the thyroid gland. The value of this suggestion is not known. It is more important to ligate the trunk of the inferior thyroid artery immediately after it leaves the carotid artery, where the procedure is harmless. It is also important that the connection between the posterior thyroid capsule and the surrounding tissues is preserved during the wedge-shaped resection of a single adenoma next to the posterior capsule of the thyroid gland and during total thyroidectomy. In this way, both the parathyroids and their blood supply remain intact.

Variation in Position. It is sometimes very difficult to find the parathyroids when they are not in the usual position. The upper glands must then be systematically searched for on the entire posterior surface of the thyroid gland, at its upper pole, between the trachea and the esophagus, and higher up, along the carotid artery. The lower glands are occasionally displaced cranially against the posterior surface of the thyroid gland, but more often distally against the jugular vein, in which case the parathyroid gland lies either in the tip of the thymus or free in the retrosternal space. The presence of a goiter makes it much more difficult to find the parathyroids. Occasionally, one gland may not only be displaced by a nodule of a goiter but also compressed and pushed out of shape into a long, flat, oval band on the surface of the nodule. In some animal species, it has frequently been reported that it is normal for the parathyroids to be completely embedded in the thyroid gland. This rarely occurs in man (p. 931, intrathyroidal adenoma of the parathyroid).

Topography of Mediastinal Adenomas. Adenomas of the parathyroid glands can be displaced into the anterior or posterior mediastinum or onto the anterior surface of the pericardium by negative intrathoracic pressure since the glands are not fixed to any cervical organs. A vascular stem is often found running towards the thyroid in such cases. The surgeon can use the vascular stem as a guide to the correct position in the retrosternal space. When a mediastinal adenoma is suspected, every downward branch of the inferior thyroid artery should be dissected out to decide whether a vascular stem of this type is present or not.

Cytology. Light microscopy allows division of the parenchymal tissue of the parathyroids into 3 cell types: the chief cells, the water-clear cells (GETZOWA), and the oxyphil cells (WELSH). Chief cells and water-clear cells are connected by numerous intermediary forms. We differentiate between small dark chief cells and large clear chief cells, and between small and large water-clear cells. The small dark and the large clear chief cells make up the main part of the normal parathyroid. The water-clear cells have strongly vacuolized protoplasm with sharply demarcated cell borders. The large water-clear cells can be 2–3 times as big as the large clear chief cells.

From the microscopic findings in inactive, active and hyperactive glands it can be concluded that parathyroid hormone is formed primarily in the large chief cells. Since these large chief cells are the predominant element in the gland of the young subject, they were also called young chief cells earlier. Other authors name them the clear chief cells or the water-clear transitional cells. The small, dark chief cell with a small nucleus appears to be the chief cell in the resting phase. The functional significance of the water-clear cells, which occur only infrequently in the parathyroids of a healthy subject, is not clear. All the chief cells as well as the water-clear cells contain large amounts of glycogen, the inactive dark chief cells containing the least and the water-clear cells the most. The pronounced vacuolar aspect of the water-clear cells, making the protoplasmic space look empty, is due to a multitude of small membrane-limited vacuoles originating from the Golgi apparatus. They do not contain glycogen.

The protoplasm of the oxyphil cells has a very strong affinity for acidic stains in particular, but also for basic substances. Some of them are found scattered diffusely in the parenchyma, and some as goups of cells or as microadenomas. Very few of these cells are found in the parathyroids of newborns and adolescents. They increase greatly in number with increasing age, and in the female after the menopause they account for up to 10% of the total mass of parathyroid glands, or 90% of a single gland. More recent electron microscope and histochemical investigations have shown that a large part of the protoplasm of these cells is composed of mitochondria closely packed together and of mitochondrial enzymes. The significance of these oxyphil cells is still largely unknown (HAMPERL).

Synthesis of parathyroid hormone, secretion granules, and cellular organelles on electron microscope examinations (MUNGER, 1963; LANGE, 1961; ROTH, 1971) have shown that parathyroid hormone, like other polypeptide hormones such as insulin and pituitary hormones, is probably found in secretory granules in the cells. The pro-secretory and secretory parathyroid hormone granules are oval and round. They are enclosed by a membrane and have a diameter of 100–220 μm. They are developed in the region of the Golgi apparatus and later released into the protoplasm, probably leaving the cells via exocytosis.

Active chief cells of the normal human parathyroid contain a well-developed Golgi zone, abundant ergastoplasm (site of protein synthesis), many secretory granules, a few mitochondria and little glycogen. Inactive chief cells of inactive glands have all the opposite features. The oxyphil cells and the uncommon water-clear cells of the normal gland contain only very few and only small secretory granules. Their Golgi zones and their ergastoplasms are difficult to demonstrate and are small. The oxyphil cells contain a large amount of mitochondria, and the water-clear cells contain much glycogen.

The parenchymal cells of the parathyroid adenoma in primary hyperparathyroidism invariably behave like hyperactive cells. They contain a great abundance of large secretory granules, as well as a strong ergastoplasm, ring lamellae, a precursor of the ergastoplasm, and a Golgi apparatus. It is striking that the cells of the oxyphil and of the water-clear adenomas behave in exactly the same way as long as these adenomas are really associated with the clinical features of primary hyperparathyroidism, which is a definite difference from the oxyphil and water-clear cells of the normal parathyroid, in which all these cellular organelles are only poorly developed.

The parenchymal cells of the hyperplastic parathyroid assume an intermediate position between the normal gland and the adenoma. This applies in particular to the secretory granules, the Golgi apparatus and the ergastoplasm. In cases of severe hyperparathyroidism with highly increased parathyroid hormone concentrations in the blood, the parathyroid cells look depleted of secretory granules, whereas in moderate hyperparathyroidism a considerable number of granules is visible. ROTH (1971) suggests that the release of parathyroid hormone can be suppressed without a concomitant decrease of the synthesis of parathyroid hormone.

Structure, Fat Cells, Colloid Cysts. The parathyroids have a trabecular-acinous structure. Small glands in the functionally inactive state show wide connective tissue septa. With increased activity, the number of the parenchymal

cells is increased, and there is usually also a marked increase in the number of individual cells, the very small chief cells developing into large chief cells or into small water-clear cells. As the activity increases, the full picture of hyperplasia of the parathyroid gland develops (p. 934). Due to this increase in the volume of the parenchyma of the parathyroid, the connective tissue septa in the hyperactive glands are lost entirely or reduced to narrow strands. The gland ultimately assumes the fully compact structure of the hyperplastic parathyroid.

Fat cells are scattered in the interstitial spaces and in the parenchyma. They are numerous in inactive glands and also in glands of obese subjects. They decrease steadily with increasing activity and are completely absent from hyperplastic glands. The adenomas of primary hyperparathyroidism are also almost entirely free of fat cells. During childhood and youth the glands contain only a small number of fat cells. The number of fat cells can be valuable in assessment of the functional state of a gland or of a glandular biopsy. It must, however, be borne in mind that the number of fat cells can vary greatly from one part of a gland to another, or from one gland to another. Furthermore, the number of fat cells can be greatly increased in the parathyroids of obese subjects, regardless of the degree of activity of these glands (WERNLY, 1946).

Not uncommonly, colloid granules are found scattered in the parenchyma of a normal, hyperplastic, or adenomatous parathyroid. True colloid vesicles with cuboid or cylindrical epithelium lining the walls are also found. In addition, there are alveoli without colloid, which can be small or large. True cysts can also be present. It is not unusual for colloid vesicles to arise in groups, giving a thyroid-like appearance. Large parathyroid cysts can only be distinguished from ordinary branchiogenic cysts if they can be related to the clinical diagnosis of primary hyperparathyroidism.

C. Physiology and Biochemistry of Calcium and Phosphate Metabolism

JAN A. FISCHER

1. Calcium Metabolism

Calcium and phosphate are the main constituents of the skeleton. Calcium is also a regulator of electrical and biochemical processes. In man, its concentration in the plasma is kept within narrow limits. As far as is known, the concentration of the ionized calcium changes little in the course of the evolution of the vertebrates. The regulation of enzyme reactions, transmission of electric impulses in the nervous system, muscular contraction, lactation, the secretion of gastric juices, and renal concentrating ability are all dependent upon the concentration of the ionized calcium in the plasma. Calcium is also necessary for blood coagulation.

Death probably results from cardiac failure in patients with a total plasma calcium above 18–20 mg/100 ml or below 4 mg/100 ml. In hypercalcemia, the ventricle is in a state of contraction at the time of death, and in the past the development of the contraction was even used as an assessment of the concentration of the ionized calcium in an unknown solution. There are at least four points of action in muscle: the conducting nerve, the muscle end plate, the electrically charged membrane of the myofibrils and the actomyosin. In addition, there are biochemical sites of action in numerous enzyme reactions. It is not known precisely what process brings about cardiac arrest in acute hypercalcemia. As a rule, the patients die of renal failure in chronic hypercalcemia.

This chapter deals chiefly with abnormal regulation and disorders of calcium metabolism. The molecular processes in which calcium is an important trace element have not been dealt with. The reader is referred to p. 856 for a discussion of its function as a supporting mineral in the bones.

a) Distribution

More than 99% of the body calcium is found in the skeleton and the teeth (p. 856). The remainder is distributed in the extra- and intracellular fluids.

Plasma. The calcium concentration is most easily measured in the plasma, and this estimation is clinically the most important. The calcium concentration in the plasma is 8.5–10.5 mg/100 ml, depending on the method used. When the same method is used the variations do not exceed $\pm 5\%$ in normal control persons, and $\pm 1\%$ in the same subject when measured at the same time of day. The concentrations are kept within the narrow limits mentioned above predominantly by parathyroid hormone and calcitonin (Table 1). In addition to parathyroid hormone, vitamin D and thyroxine cause a rise in the serum calcium concentration, whereas calcitonin, cortisone and glucagon cause a fall. A deficiency of parathyroid hormone may be present if recovery of the normal calcium concentration in the plasma is delayed after an intravenous infusion of EDTA, which

chelates calcium. The rise of the calcium concentration in the plasma is accelerated by the administration of growth hormone, which presumably causes increased calcium mobilization from bone (GERSHBERG, 1967).

Calcitonin causes a fall in the calcium concentration in the plasma. Calcitonin deficiency arises in patients after thyroidectomy, and these patients show a delayed return of the calcium concentration to normal values after intravenous administration of calcium (MAZZUOLI, 1966). Cortisone also causes a fall in the serum calcium concentration. One exception to this, however, is the elevated calcium concentration in the plasma in hyperparathyroidism. This difference provides a way of differentiating hypercalcemia due to hyperparathyroidism from that due to other factors (DENT, 1962) (p. 926).

Table 1

Factors increasing the serum calcium concentration
Parathyroid hormone
Vitamin D (oversensitivity in sarcoidosis)
Calcium
Thyroxine
Growth hormone
Vitamin A
Paraneoplasia (parathyroid hormone-like substances)
Bone metastases (predominantly osteolytic)
Factors lowering the serum calcium concentration
Calcitonin
Cortisone
Phosphate
Vitamin-D deficiency (? resistance)
Paraneoplasia (?)
Bone metastases (predominantly osteoplastic)
Glucagon

A normal serum calcium concentration can only be attained in the presence of a minimum intake of vitamin D and/or of calcium, and of a maximum intake of phosphate. An increase of the plasma phosphate is associated with a fall in the concentration of the serum calcium. Parathyroid hormone and vitamin D (and its metabolites) are necessary for regulation of the serum calcium concentration, and their release into the plasma is in turn dependent on the concentration of the ionized calcium (p. 871). Methods for determining ionized calcium are not yet generally applied, since measurement of the total calcium concentration is usually sufficient for clinical purposes. The concentrations of diffusible and ionized calcium are dependent on the pH and on complexing agents such as phosphate, citrate, other organic anions, and variations can thus remain unobserved if only the total calcium concentration is measured. Conversely, variations in the concentration of serum proteins lead to hyper- or hypocalcemia in the presence of unchanged concentrations of ionized calcium. This occurs in an extreme form in the hen, where the serum calcium can rise to 30 mg/100 ml in the presence of an ionized calcium concentration of about 6 mg/100 ml. Of the calcium in the serum, 60.5% is in a diffusible form (46.9% ionized, 13.6% as bicarbonate, phosphate and citrate complexes, and 39.5% bound to proteins (MOORE, 1970) (Fig. 1). Similar values can be found in WALSER (1961). The differential diagnosis between hyper- and hypocalcemia is described on p. 926. The calcium concentration in the extracellular fluid is 7 mg/100 ml. In the *cerebrospinal fluid*, it is 4.5–6 mg/100 ml. These values correspond approximately to the concentration of ionized calcium, since the calcium bound to the plasma proteins cannot diffuse freely.

The concentration of intracellular calcium can only be roughly estimated. In the liver and the kidney, calcium is found predominantly in subcellular particles. The calcium in the cytoplasm, defined as cell sap free of organelles, amounts to 10^{-6} to 10^{-7} M (THIERS, 1957). There is a steep concentration gradient between the extracellular fluid and the cytoplasm. Calcium is stored intracellularly in the mitochondria, the microsomes and the cell nuclei. No absolute concentrations can be given for the intracellular fluids because of the inhomogeneous distribution in the cell, and there is no point of reference, particularly since the volume, the pH, and the concentrations of the proteins and anions of the individual compartments cannot be measured accurately. As a rule, the concentrations are deduced from the nitrogen content or the

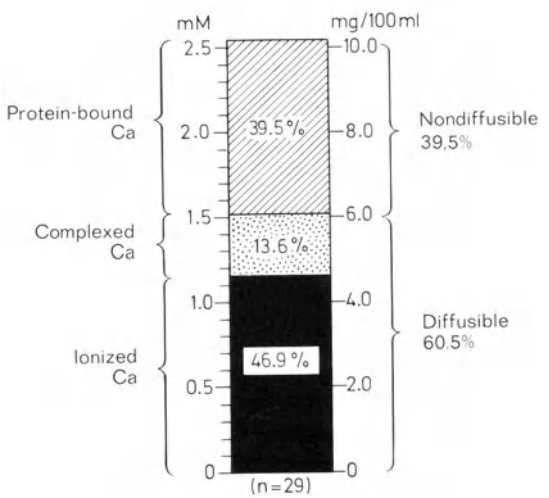


Fig. 1. Fractions of total serum calcium as obtained by ultrafiltration and determination of the ionic calcium concentration in 29 normal sera (percentages are mean values of the group). (After MOORE, 1970)

nucleic acid content of the cell or from the subcellular particles. In addition, there is an inhomogeneous distribution in individual organs and it is difficult to obtain an insight into the complex distribution and compartmentalization of calcium. It is easier to measure the calcium concentration in erythrocytes, which contain only small amounts (0.634 µg/ml) (HARRISON, 1968). Myocardial function can partially be judged by the ECG since the Q-T duration varies inversely to the serum calcium concentration.

Hypercalcemia and a rapid intake of large amounts of phosphate can result in nephrocalcinosis and in the calcification of organs and blood vessels. The calcifications arise preferentially in alkaline regions in the kidneys and occasionally in the gastric mucosa. Furthermore, calcifications arise in necrotic areas, and possibly for the same reasons also in the liver following poisoning with carbon tetrachloride. Calcification of the basal ganglia is found in idiopathic hypocalcemia (p. 899). DANOWSKI (1962) gives a detailed account.

b) Balance

Calcium is absorbed from the small intestine and is excreted in the gut, urine and sweat. It is also excreted through the placental circulation and in the milk (Fig. 2). The determination of the calcium balance is inexact and time-consuming since an equilibrium between the multiple compartments of the bone with variable calcium intake can be obtained only over a period of weeks and months.

α) Calcium Intake

The daily requirement of calcium is about 0.5–1.0 g for the adult, 1.0–1.5 g during growth and pregnancy, and at least 1.5 g during lactation. The daily intake is 0.5–1.5 g in West European countries and in North America. Intake may very well be below the minimum requirements, especially in advanced age and during pregnancy, and it is certainly below the minimum in some developing countries.

With an increased nutritional intake of calcium, the amount of calcium absorbed in the small intestine decreases steadily, as the intestinal mucosa becomes saturated. Maximum absorption is achieved with an intake of 3–5 g/24 h, and is 1–1.5 g/24 h (NORDIN, 1968).

Calcium Absorption. Calcium absorption is limited to the upper small intestine, the duodenum and the jejunum. The methods available for the measurement of calcium absorption are preeminently of academic interest. The simplest method, which is rather inaccurate, is limited to the comparison of the calcium intake and the fecal calcium excretion. The calcium intake can easily be measured providing the calcium content of the drinking water is considered in the calculation. Measurement of the calcium excretion in the stools is time-consuming and must be carried out over balance periods of 3–7 days. Chromium is given constantly to the patient by mouth and the calcium excretion is related to the chromium content in the stools (HARGREAVES, 1965). With a daily intake of 1 g, or 15 mg/kg body weight, the fecal excretion is about 700 mg or 11 mg/kg body weight (Fig. 2). The error in the measurement can be as high as 1000 mg/24 h in isolated cases, and the measurements sometimes have to be continued over 4-day periods for over 100 days (ISAKSSON, 1967). This method is not suitable for determining differences of less than 100–200 mg/24 h in the balance.

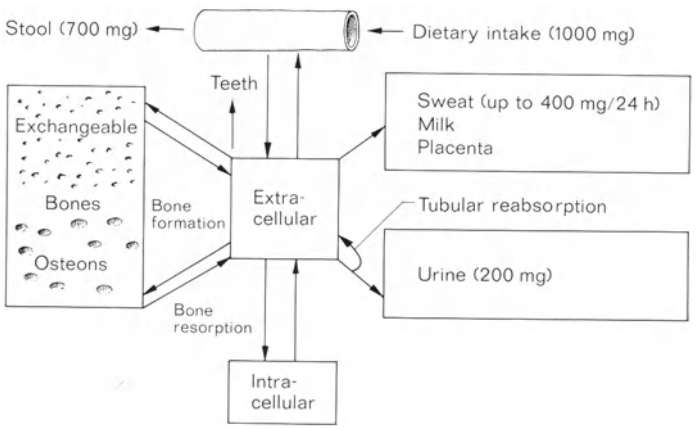


Fig. 2. The metabolism of calcium. The schematic representation of the osteons and the exchangeable calcium pool reflects the autoradiographic picture. For explanations see text.

Calcium absorption is estimated more simply with radioactive ^{47}Ca after oral intake of 5–20 μCi ^{47}Ca (half-life 4.7 days). The rise in the concentration can be measured in the plasma, or the retention is determined with a whole-body counter or with a scintigraph over specific parts of the skeleton such as the radius (AVIOLI, 1965; SODE, 1968). This method is easy to use. Measurement of the specific activity in the plasma has the disadvantage that it is impossible to allow for secretion through the intestine, the calcium excretion in the urine, the plasma volume and the flow rate of the calcium from the plasma to other compartments of the body, especially in pathologic cases. With the whole-body counter method and scintigraphy, a homogeneous distribution in the skeleton is assumed. This disadvantage can be counteracted by using radioactive ^{47}Ca in combination with ^{45}Ca, which has a longer half-life. Both isotopes are administered orally and intravenously at the same time. The ratio of the two isotopes excreted in the urine is measured 24 hours later, and the amount of calcium absorbed from the small intestine is calculated. The rapid decay of ^{47}Ca and the formation of the less energy-rich ^{47}Sc must be taken into account in the measurement of ^{47}Ca and correspondingly screened off (DE GRAZIA and RICH, 1965).

Transport mechanisms of calcium through the intestinal mucosa are dealt with in the section on vitamin D (p. 882). In the intestine, calcium deficiency leads to increased absorption of magnesium. Magnesium deficiency results in increased calcium absorption (ALCOCK, 1962). It is still uncertain whether there are transport routes common to both magnesium and calcium through the intestinal mucosa of whether magnesium is simply a requirement for calcium absorption. Magnesium promotes calcium absorption in the intestine and leads to elevated retention of calcium and magnesium in the bones (CLARK, 1967, 1968). Extreme magnesium deficiency leads to hypocalcemia in rats (MCMANUS, 1969). Intestinal calcium absorption and calcium release from the bones are dependent on a minimum magnesium concentration.

Increased Calcium Absorption. Vitamin D, parathyroid hormone and growth hormone promote the absorption of calcium. It is also increased in the presence of an acid pH, by an increase in the ionized calcium concentration, and by lactose. A protein-rich diet with increased amounts of basic amino acids, pregnancy, the growth period, calcium-deficient nutrition and phosphate deficiency also promote calcium absorption. Calcium absorption is increased in sarcoidosis, possibly due to elevated sensitivity to vitamin D. The effects of vitamin D and parathyroid hormone occur only after some hours, whereas inhibition of calcium absorption by intravenous calcium infusions can be measured within minutes (BIRGE, 1969).

Reduced Calcium Absorption. Calcium absorption is reduced in the presence of adrenocortical steroids, thyroxine, vitamin-D deficiency and rickets. It is also reduced in the malabsorption syndrome with steatorrhea due to the reduced absorption of vitamin D and of calcium. Calcium absorption is diminished in hyperthyroidism, in hypoparathyroidism, and after administration of alkalis, oxalate, phytate or phosphate. In inflammatory processes in the region of the intestine, calcium absorption is sometimes increased after the administration of cortisone, if it influences the basic disease.

β) Calcium Excretion

Calcium is excreted into the intestine, and in the urine, sweat, and breast milk.

Intestine. The daily excretion into the intestine is 150–450 mg or 2–6 mg/kg body weight, and is approximately the same as the calcium excretion in the urine.

Calcium excretion in the feces is measured after the intravenous injection of ^{47}Ca. Assuming that the specific activity ($^{47}Ca/^{40}Ca$) of the calcium excreted in the urine is the same as that of the calcium excreted in the stools, the excretion of the total calcium in the stools can be calculated from the excretion of the ^{47}Ca and ^{40}Ca in the urine and from the radioactive ^{47}Ca in the feces (LAZOR, 1963).

Kidneys. The urinary calcium excretion is dependent on the calcium concentration of the ultrafiltrable calcium (about 60 % of the total calcium) in the glomerular filtrate and on the tubular reabsorption of calcium. Calcium excretion is increased by an increased nutritional intake and/or intestinal absorption such as occurs in sarcoidosis, and by an elevated bone breakdown such as occurs in hyperthyroidism and in acidosis. The urinary excretion is also increased in hyperparathyroidism because of a direct inhibition of calcium reabsorption in the proximal tubules. Small differences in the ultrafiltrable calcium can lead to considerable variations in the urinary calcium without remarkable changes of the total serum calcium concentration.

Calcium is filtered through the glomeruli, and about 99 % is reabsorbed in the tubules.

Providing renal function is normal, the excretion threshold through the kidneys lies at a serum concentration of about 8 mg/100 mg. When the serum concentration falls below 8 mg/100 mg, practically all the calcium is reabsorbed and less than 30 mg is excreted in 24 hours. With normal nutrition, the daily excretion is 150–450 mg, or 2–6 mg/kg body weight. With a calcium-free diet the excretion is below 200 mg. The maximum excretion in the urine is about 1 g/24 h. When this concentration is exceeded, calcium is deposited as renal stones or in the tissue until the glomerular filtration is so limited that calcium can no longer be excreted in large amounts. In such cases it is not always possible to determine whether primary hyperparathyroidism was originally present or whether the results are due to secondary hyperparathyroidism, resulting from the hypocalcemia of renal insufficiency, or even autonomic tertiary hyperparathyroidism. Since the calcium clearance never exceeds the creatinine clearance, secretion of calcium in the tubules cannot be confirmed or excluded. The tubular reabsorption, like the absorption in the small intestine, is promoted by parathyroid hormone and inhibited by cortisone (LAAKE, 1960).

Calcium, magnesium and sodium are presumably reabsorbed in the renal tubules by a common carrier mechanism. The details are unknown. Calcium infusion leads to increased excretion of magnesium (COBURN, 1967). Increased magnesium intake results in an elevated excretion of calcium in the urine, which may be due partly to increased calcium absorption in the intestine (CLARK, 1968). The micropuncture method has been used to demonstrate that calcium and sodium are reabsorbed in the same region of the proximal tubule (DUARTE, 1967).

Calcium and magnesium reabsorption is diminished by an increased sodium intake with an expansion of the extracellular space, and the excretion into the urine is increased, while calcium excretion is decreased in response to a diminution of the extracellular space by thiazides. Administration of desoxycorticosteroids such as 9-fluorohydrocortisone with simultaneous intake of sodium leads to expansion of the extracellular space and only then to an increase in the excretion of calcium and magnesium into the urine (MASSRY, 1968; SUKI, 1968). The tubular reabsorption of calcium is maximally increased in hyperparathyroidism. An additional loading of the renal tubules by an infusion of sodium chloride, leads to a rise in the calcium excretion, in contrast to the result in normal control subjects (AXELROD, 1966). A sodium-deficient diet, however, causes an increase in the tubular reabsorption of calcium in idiopathic hypercalciuria (EDWARDS, 1965).

Calcium excretion in the urine is increased in the presence of complexes such as citrate, in phosphate deficiency, and in acidosis. Furthermore, calcium excretion is increased by saliuretics such as ethacrinic acid, in osmotic diuresis and by carbohydrates. Carbohydrates produce a higher calcium excretion in patients with calcium oxalate stones and in their relatives than in control subjects (LEEMAN, 1969).

Hypercalciuria can result in nephrocalcinosis and nephrolithiasis, but calcium is not the only factor involved in these disorders. Stone formation is favored by an alkaline pH, which is usually found in hyperparathyroidism, in Cushing's syndrome, in primary hyperaldosteronism and in urinary tract infections. Renal stones are most frequently found in hyperparathyroidism, in idiopathic hypercalciuria, in Paget's disease, in Cushing's disease and in immobilization osteoporosis, which may also be found with weightlessness in astronauts.

Calcium and phosphate are present in the urine in an oversaturated solution, so that protective substances which prevent the formation of renal stones must be assumed. Phosphonates, which differ from pyrophosphate in that they cannot be metabolized by renal pyrophosphatases, inhibit nephrocalcinosis (FLEISCH, 1968). A different substance is a peptide, which has not yet been successfully purified (HOWARD, 1968).

Hypercalciuria leads to water diuresis with diminished water reabsorption and to increased excretion of sodium in the urine. Inhibition of water reabsorption in the ascending Henle's loop can be assumed (BINSWANGER, 1967; SUKI, 1969). Selective perfusion of the proximal and distal tubules of the rat with calcium inhibits water reabsorption in the distal tubules. These are morphologically altered in dog following calciuria, which explains vasopressin resistance (LASSITER, 1965; EPSTEIN, 1959).

Hypocalciuria develops in particular with a fall in the calcium concentration in the serum, and with diminished calcium uptake in the intestine, in intestinal malabsorption syndromes or following oral administration of phytates and phosphate. Thiazides and a sodium-deficient diet lead to increased reabsorption of calcium by the renal tubules. These drugs are therefore used for the treatment of idiopathic hypercalciuria (YENDT, 1966).

Sweat. The calcium excretion is 20–365 mg/24 h, depending on the temperature and the humidity

of the air. The average excretion is 120 mg/24 h (ISAKSSON, 1967). Excretion in the sweat is measured on the arm and is related to the potassium excretion, which in turn is calculated from the exchangeable potassium and the potassium balance of the total body surface. The calcium concentration varied, depending on the author, between 0.3 and 15 meq/liter. Between 0.1 and 0.7 meq/liter, it is mainly proportional to sweat excretion (VELLER, 1968).

Fetus. Calcium excretion through the placenta corresponds to the calcium content of the fetus, which contains 30 g calcium and 22 g phosphorus at term. With a calcium content of 1200 g in the adult, the 30 g correspond to a loss of 2.5% during pregnancy. This is partly compensated for by a greater calcium absorption in the intestine with a corresponding rise in the parathyroid hormone concentration (CUSHARD, 1972); during the last quarter of pregnancy, about 250 mg calcium is incorporated daily into the fetus (CUSHARD, 1971; HYTTEN, 1964).

Lactation. About 50–70 g calcium is excreted in the milk. When lactation is prolonged about 500 g is excreted. The World Health Organization therefore recommends a daily calcium intake of 1200–1500 mg between the fourth and ninth months of pregnancy and about 1200–2000 mg during lactation.

Summary. Determination of the calcium balance is inexact and time-consuming, particularly because the fecal excretion and the loss in the sweat are not easily determinable. After an alteration of the calcium intake, weeks or months may be required before equilibrium between the different compartments of the bone is attained. Intestinal calcium absorption can be estimated with isotopes, but this only permits comparisons between patient groups with increased, diminished or normal calcium absorption. The most important single measurement is the estimation of the serum calcium concentration.

2. Phosphate Metabolism

Phosphate and calcium are the main bone minerals. Phosphate is found in higher concentrations than calcium in the intracellular fluid where it is an important anion and component of the nucleic acids and of the energy-rich compounds such as adenosine triphosphate. Phosphate is a regulator of oxidative phosphorylation and a buffer in cells, plasma and urine.

a) Distribution

About 80% of the phosphate is found in the bones and teeth. The remainder is distributed throughout the intra- and extracellular fluids, and half of it is found in the musculature.

Plasma. In contrast to the calcium concentration in the plasma, which is kept within the same narrow limits throughout life, the phosphate concentration in the plasma is age-dependent. In the adult the concentration of inorganic phosphorus is 2.5–4.5 mg/100 ml. During youth, it is 4.0–7.0 mg/100 ml, and it rises to 4.5 mg/100 ml during the menopause. Of the inorganic phosphate in the plasma, 88% is ultrafiltrable (WALSER, 1961). Phosphate acts as a buffer substance in the plasma and the urine, although the total buffering capacity in the blood is only 5.5% (ELLISON, 1958). In addition to inorganic phosphate, there is about 1 mg/100 ml phosphorus in the form of phosphate esters and 8 mg/100 ml as phosphates bound to lipids. The total phosphorus concentration in the plasma is about 12 mg/100 ml. The concentration of inorganic phosphate in the plasma shows considerable diurnal variations and is dependent on nutritional intake (Fig. 3) (STANBURY, 1958). The serum phosphate is lowest in the morning and highest in the evening. This rhythm is approximately parallel to fluctuations in the concentration of parathyroid hormone in the serum (ARNAUD, 1971). The normal rhythm of the phosphate concentration in the plasma and of the phosphate excretion in the urine disappears after removal of the adrenals or of the pituitary gland. The changes

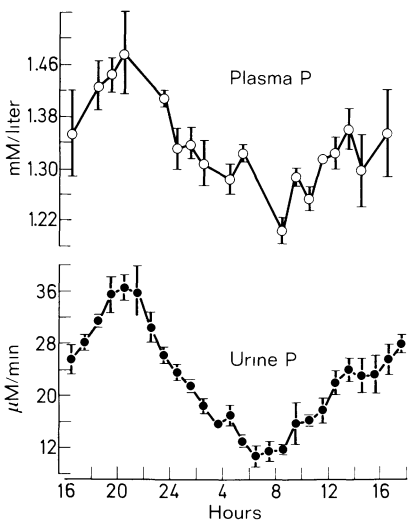


Fig. 3. Diurnal variation of the serum phosphorus concentration and the urinary phosphate excretion in three normal subjects. (After STANBURY, 1958)

in the serum are paralleled by those in the urine and cannot therefore be related to changes in the urinary phosphate excretion. There may be some interrelationships between the diurnal fluctuations of the cortisol levels and of the phosphate levels in the plasma (GOLDSMITH, 1965). The phosphate concentration is measured in the morning in the fasting state. Serum proteins and erythrocites must be separated within two hours of the blood collection, so that organic phosphates are not measured as well.

The phosphate concentration in the plasma is influenced by the nutritional intake of phosphate, by intestinal absorption, by the incorporation and release of phosphate in the bones and in the intracellular fluid, and by its excretion through the kidneys. Vitamin D, parathyroid hormone, thyroxine and acidosis accelerate bone metabolism and thereby the release of calcium and phosphate into the plasma. Growth hormone promotes the reabsorption of phosphate in the renal tubules and leads to an elevated phosphate concentration in the serum during growth and in acromegaly (CORVILAIN, 1964). Parathyroid hormone and calcitonin reduce the serum phosphate concentration by increasing the excretion through the kidneys. Furthermore, calcitonin inhibits bone resorption, and insulin and glucose increase the uptake of phosphate into the intracellular fluid. For reasons which have still not been explained, the serum phosphate concentration is diminished in the presence of septicemia caused by gram-negative organisms (RIEDLER and SCHEITLIN, 1969).

The differential diagnosis of hyper- and hypophosphatemia is discussed on p. 940.

The concentration of inorganic phosphate in the *extracellular fluid* corresponds to the serum concentration.

It is 1–2 mg/100 ml in the *cerebrospinal fluid*, where it is increased in hypoparathyroidism.

Most of the phosphate in the *intracellular fluid* is in an organic form. Phosphate is a component of nucleic acids, of energy-rich compounds such as adenosine triphosphate (ATP), hexose phosphates, and creatine phosphate. Transport of phosphate through the intracellular membranes, such as the mitochondrial membrane, is increased under the influence of parathyroid hormone. Phosphate acts as a regulator of ATP synthesis and of additional energy-rich compounds which, together with oxidative phosphorylation and the respiratory chain, facilitate the transport of ions through the mitochondrial membrane. ATP is an important source of energy for muscular contractions, for the synthesis of cell constituents and for glandular secretions. Phosphate deficiency leads to muscular weakness, nausea, and anorexia (LOTZ, 1968).

In *the urine*, phosphate forms an important buffering system for the excretion of hydrogen ions, after bicarbonate and ammonia. Phosphate is excreted predominantly as $H_2PO_4^-$ at pH 4.8, and as HPO_4^{--} at pH 7.8.

b) Balance

Phosphate is absorbed in the intestine and is incorporated into the bones and the intracellular fluid. About 80% is excreted in the urine and sweat. Some is presumably excreted into the intestine.

α) Phosphate Intake

The daily requirement of phosphate corresponds to that of calcium and is about 1 g (calculated as phosphorus). Only organic phosphates are absorbed. Elemental phosphorus is highly toxic.

Absorption. The regulatory mechanism for the absorption of phosphate in the intestine is not well known. Phosphate is presumably absorbed by passive diffusion. Vitamin D, parathyroid hormone and growth hormone accelerate the absorption of calcium and thus also that of phosphate. These substances presumably have no direct effect on the absorption of phosphate.

Increased absorption is found with a calcium-deficient diet, following the administration of fats and of cations, and in the presence of an acid pH.

Reduced absorption is found with reduced calcium absorption or a predominance of cations such as calcium, strontium, magnesium and aluminum, which form complexes with phosphate. Aluminum hydroxide gel is used for the treatment of renal insufficiency to reduce phosphate absorption. Organic phosphate compounds such as phytates cannot be hydrolyzed and therefore cannot be absorbed.

β) Phosphate Excretion

Phosphate is excreted in the urine, stool and sweat. Further, about 22 g phosphorus are incorporated into the fetus during pregnancy. The loss in the maternal milk during a lactation of average duration is about 40 g.

Kidneys. Like the plasma concentration, the excretion of phosphate demonstrates a circadian rhythm which is less obvious in the absence of parathyroid hormone. The phos-

phate clearance must therefore always be measured at the same time of day or, better still, over 24 hours (STANBURY, 1958) (Fig. 3). Theoretically, the most accurate measurement of the phosphate excretion is obtained by measuring the maximum tubular reabsorption (T_{mP}) while the phosphate intake is changed by means of intravenous infusions of phosphate. This investigation is complicated by the fact that infusions of phosphates cause the ionic calcium concentration to fall within minutes, with a corresponding increase of the parathyroid hormone concentration and a reduction of the T_{mP} (FISCHER, 1973). Furthermore, a potassium loss may also be produced, which in itself causes a reduction of the T_{mP} (STANBURY, 1958). The phosphate excretion is measured primarily as the tubular reabsorption (TRP) or as the maximal tubular phosphate reabsorption (BIJOVET, 1970). Phosphate is filtered through the glomeruli and about 85–95% is reabsorbed in the tubules. When serum phosphate levels are increased with phosphate infusions, even in hypoparathyroidism, only 38% is reabsorbed (FAIRHURST, 1963). The ratio of the phosphate clearance to the creatinine clearance gives an indication of the tubular phosphate reabsorption (TRP) (the calculation is given on p. 944). The maximum tubular phosphate reabsorption can be measured directly, or, more conveniently, read from a nomogram (BIJOVET, 1969).

There is probably a transport mechanism common to glucose and phosphate in the proximal renal tubules. Increased amounts of glucose are reabsorbed under the influence of parathyroid hormone. An increase in the maximum glucose reabsorption (Tm) is thus interpreted as a sign of hyperparathyroidism in the differential diagnosis of hypercalcemia (TRANSBOL, 1967).

Apart from being reabsorbed in the proximal tubules, phosphate is also secreted in the distal tubules in amphibians and in birds. This is also true of man during the administration of high doses of phosphate, when phosphate clearance may exceed the glomerular filtration rate (WEBSTER, 1967).

The excretion of phosphate in the urine is dependent on the nutritional intake, on the plasma concentration, and on factors which influence the tubular reabsorption or secretion. Renal phosphate excretion is increased by various factors: parathyroid hormone, calcium administration, cortisone, estrogens, thyroxine, increased protein intake, hypokalemia, hypomagnesemia, acidosis, bicarbonate administration and expansion of the extracellular fluid volume (SUKI, 1969). As a rule, the excretion of phosphate in the urine is elevated in primary hyperparathyroidism, providing renal function is not seriously impaired. It is elevated in secondary hyperparathyroidism, in rickets, in osteomalacia, in hyperthyroidism, in acidosis (intensive muscular activity, diabetes, hunger), with the intake of large amounts of phosphate, in gout, in acute immobilization osteoporosis, in the presence of predominantly osteolytic bone metastases, especially after treatment with estrogens, in vitamin-D intoxication, in sarcoidosis, in tubular lesions (renal tubular acidosis, pyelonephritis) and in vitamin D-resistant rickets. The urinary excretion of phosphate is difficult to evaluate in certain cases for the diagnosis of hyperparathyroidism, since it can be elevated in all forms of hypercalcemia.

In uremia renal osteopathy develops, which is associated with a decrease in the serum calcium concentration. This leads to secondary hyperparathyroidism with a reduction in the tubular phosphate reabsorption. The urinary phosphate excretion is only suppressed in the presence of a greatly reduced glomerular filtration rate (FALLS, 1966) (p. 871).

Angiotensin diminishes the excretion of phosphate, sodium, chloride, calcium and magnesium, presumably by inhibiting the renal blood flow (BRODEHL, 1966). The excretion of phosphate is reduced by growth hormone, insulin and hydrocortisone. Thus, it is decreased during growth and in hyperinsulinism. It is also reduced during pregnancy and lactation, vitamin-D deficiency and intestinal malabsorption, with reduced renal function, in hypoparathyroidism, after parathyroidectomy, in certain cases of idiopathic hypocalcemia, in Addison's disease and in the presence of predominantly osteoplastic bone metastases.

A small proportion of the phosphate is excreted in the urine in the form of pyrophosphates which probably originate from the bones (p. 889). The excretion of phosphate, pyrophosphate and hydroxyproline is correspondingly elevated in hyperparathyroidism (AVIOLI, 1966).

Sweat. Phosphate excretion in the sweat has not yet been directly measured. The calcium and nitrogen excretion is estimated in the sweat. The phosphate excretion is estimated on the assumption that the ratio between the phosphate and calcium or nitrogen excretions does not differ from the whole body balance. The excretion in the sweat is 125 mg/day on average (ISAKSSON, 1967).

Intestine. The amount of phosphate presumably secreted together with calcium into the intestine is not known.

3. Bone Metabolism

a) Structure and Biochemistry of Bones

The skeleton consists of cells, the organic bone matrix (mainly collagen, mucopolysaccharides) and the bone mineral (chiefly calcium and phosphate). When bone is formed, bone matrix is synthesized and calcium phosphate is subsequently deposited. Amorphous calcium phosphate is transformed into hydroxyapatite. The skeleton consists of inorganic bone mineral (about 70%) and of the organic bone matrix (about 30%).

Macroscopically the bones are composed of *spongiosa* and *compact* parts. The spongiosa consists of trabeculae, and the compact bone of concentric lamels. It is found in the subperiostal regions of the skeleton and in the diaphyses of the long bones. The segmentation in *osteons* is characteristic of the structure of the compact bone. These osteons are histogenetically, trophically and mechanically determined units. They consist of a narrow Haversian canal layered with osteoblasts, and undifferentiated cells, arteries, capillaries, veins and nerve fibers. Around the Haversian canal the bone substance is deposited in concentric lamels. Between these layers the osteocytes are found. The reader is referred to the appropriate textbooks for the detailed description of the anatomy of the bones.

α) Bone Cells

Bone is probably separated from the extracellular fluid by a cellular membrane. NEUMANN (1969) found six times more potassium in the so-called bone-water than in the extracellular fluid. The exchange of fluids, salts and nutrients is regulated by cells which control the formation and the resorption of bone.

The osteocytes orignate from osteoblasts which, in turn, are formed from undifferentiated mesenchymal cells. This series of events can be demonstrated autoradiographically with the aid of thymidine labeled with tritium. It can be shown that thymidine is incorporated into desoxyribonucleic acids and cell division occurs in undifferentiated mesenchymal cells, from which osteoblasts and osteocytes, and osteoclasts develop.

The osteoblasts are only present in growing osteons. They are responsible for the formation of new bone. The cytoplasm of the osteoblasts is rich in ribonucleic acids which regulate protein synthesis. They also contain large amounts of enzymes such as the alkaline and acid phosphatases which can be demonstrated histochemically. The osteoblasts excrete the organic *bone matrix* and have a well-developed Golgi apparatus (YOUNG, 1964). Increased osteoblastic activity can be demonstrated in the presence of a rise in the concentration of the *alkaline phosphatase* in the plasma. It runs parallel to an increase of the accretion as measured by isotopic-kinetic analysis (p. 863). There is no correlation with bone resorption, however (KLEIN, 1964).

It is possible to separate the alkaline phosphatases from the placenta, the leukocytes, and the small intestine on electrophoresis. Alkaline phosphatase of the bone – but not of the liver – is inactivated by heat. For practical purposes the results are disappointing (POSEN, 1967). The heat-stable alkaline phosphatase is slightly elevated during pregnancy.

When the liver function is normal, an elevated serum alkaline phosphatase concentration serum usually indicates increased osteoblastic activity. A familial deficiency of alkaline phosphatase (*hypophosphatasia*) leads to impaired bone formation with a pronounced increase in osteoid. This deficiency shows that an alkaline phosphatase is important in bone formation (BARTTER, 1966). *Hyperphosphatasia* or familial osteoectasia is associated with a pronounced increase in osteoblasts and in bone formation, as demonstrated by means of labeling with tetracycline (THOMPSON, 1969). The mineralization of the bones is also inadequate.

Immature osteons are surrounded by osteoblasts, whereas mature osteons are enveloped by endosteal lining cells. Osteocytes are found between the lamellae of the compact bone. They are connected by means of long processes passing through narrow canals. As in the case of the osteoblasts, it has been demonstrated histochemically that alkaline phosphatase and numerous hydrolases are present in the osteocytes. No acidic phosphatase has been demonstrated. The osteocyte is not very different from the osteoblast from which it originates (VAES, 1967). Osteocytes regulate new bone formation and breakdown (BELANGER, 1969), while plurinuclear osteoclasts stimulate bone resorption (Fig. 6a, p. 860).

β) Bone Matrix

90–95% of the bone matrix consists of collagen, a protein which contains amino acids, predominantly glycine (about 30%), proline (12%), and hydroxyproline (10%). The osteoblasts initiate the synthesis of the collagen. Free amino acids are taken up by the osteoblasts and are deposited on the ribosomes, where protocollagen is formed. At this point only proline incorporated into the protocollagen is hydroxylated into hydroxyproline. This reaction requires the presence of ascorbic acid. The protocollagen is

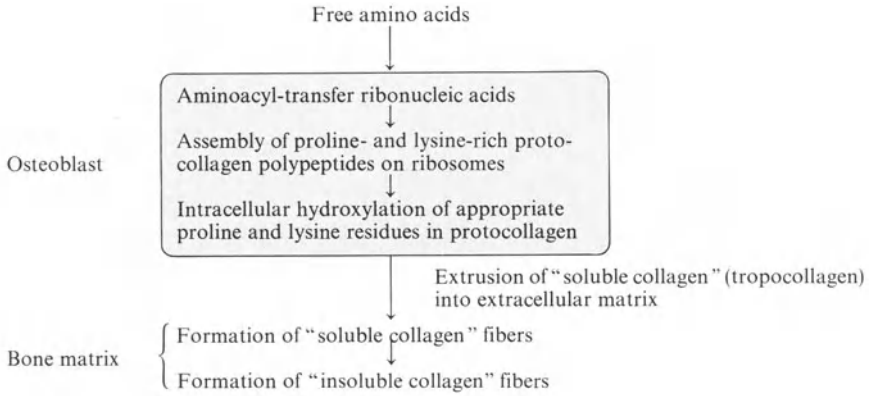


Fig. 4. The biosynthesis of collagen in the osteoblasts and in the bone matrix. (After PROCKOP, 1967)

secreted into the intercellular fluid where it is first converted into "neutral salt-soluble" and then to "insoluble" collagen fibrils which are then connected to form a network (PROCKOP, 1967) (Fig. 4). Collagen is composed of three helical polypeptide chains with a molecular weight of over 350000. Amino acids such as glycine and proline, which have been demonstrated autoradiographically, are the main components of collagen. Proline is first selectively incorporated into the osteoblasts within 30 min. From there it passes into the unmineralized osteoid within 4 hours, and appears later in the mineralized bone matrix (CARNIERO, 1959). In addition to collagen, 1–2% of the bone matrix is formed by the *ground substance* of the bone, which is made up predominantly of mucopolysaccharides such as chondroitin sulfate.

The architecture of the matrix probably determines the size of the skeleton and the orientation of the bone crystals.

γ) Bone Mineral

The main bone minerals are calcium and phosphate. They are present in the form of amorphous calcium phosphate and of hydroxyapatite (POSNER, 1969). Tetracycline is deposited in the transitional zones between osteoid and apatite. The area of the surface marked with tetracycline gives an indication of the rate of the mineralization of the skeleton and thus of the formation of new bone (FROST, 1963) (p. 860). Furthermore, the bones contain small amounts of carbonate (6%), nitrate (1%), sodium (0.7%), magnesium (0.7%), and fluoride (traces) (NEUMANN, 1958). The apatite crystals measure about 50–100 × 200–250 × 450–500 Å. They correspond to synthetic hydroxyapatite in X-ray and infra-red spectroscopy. The combination of the phosphate with the side chains of collagen molecules appears to be important for the beginning of the nucleation process (GLIMCHER, 1962). The details of the precipitation of calcium and phosphate in the net of collagen fibrils are not definitively known. Calcium phosphate is normally precipitated on the collagen of the bones and in the teeth. It is only visible macroscopically in organs such as the liver and the spleen, in the blood vessels or the skeletal musculature in pathologic conditions. The reason for this is only partially known. FLEISCH (1961, 1968) has shown that pyrophosphate plays some part in the process. Pyrophosphate *in vitro* inhibits the precipation of calcium phosphate on apatite crystals (FLEISCH, 1961) and it has been demonstrated in the plasma and in the bone. Administration *in vivo* prevents calcification of the skin and of the aorta (FLEISCH, 1966a; SCHIBLER, 1968; CARTIER, 1959). Alkaline phosphatases are also pyrophosphatases in most tissues, and particularly in the skeleton (RUSSELL, 1968). It is possible that a pyrophosphatase in the bone favors the accelerated breakdown of pyrophosphate which inhibits calcification. Precipitation of calcium and phosphate can then occur (FLEISCH, 1968).

A normal serum calcium and/or phosphate concentration is necessary for bone mineralization. NEUMANN (1958) showed *in vitro* that calcium and phosphate are present in the serum in a solution oversaturated with hydroxyapatite, and that precipitation is therefore favored. Calcification is impaired if calcium and phosphate are decreased. A fall in the calcium concentration in the extracellular fluid is observed in the presence of an increased bone formation, as for example in rachitic children treated with vitamin D, in whom the skeleton suddenly becomes more mineralized. About 500 mg calcium daily are incorporated from the extracellular fluid into the bones and correspondingly about the same amount of old bone is resorbed. In addition, there is a constant exchange of

calcium between the apatite lying on the bone surface and the extracellular fluid. These two exchange processes were estimated separately over more than 30 years with autoradiographic techniques involving exposure to ^{226}Ra or ^{90}Sr. Radioactive ^{226}Ra is bound, to a great degree irreversibly, to the skeleton (ROWLAND, 1960). The "hot spots" are clearly visible and correspond to the areas of bone formation already formed at the time the isotopes were taken up by the body. At the same time, radioactive radium is diffusely distributed over the entire surface of the bone minerals. Most of the diffusely distributed radium is presumably exchanged independently of the metabolism of the bones. The sum of the calcium ions in the extracellular fluid and in the bones remains relatively constant at least for short periods of time. About 50% of the radium is diffusely distributed after 20–30 years (MARSHALL, 1959; ROWLAND, 1960).

With advancing age, apatite replaces water in the skeleton so that bones ultimately contain no more than 3–5% water.

b) Formation and Resorption of Bone

Hormones and/or vitamin D regulate bone formation and resorption via cellular mechanisms which, in turn, regulate mineralization and demineralization. The formation of new bone and its resorption occur simultaneously in different parts of the bone. Bone remodeling is the sum of both processes, which are frequently both increased or reduced. They are regulated by hormones and require the presence of vitamins A and D, ascorbic acid, and an adequate protein intake (p. 888) (Table 3).

Bone Formation. The formation of new bone in the embryo is preceded by the formation of cartilage (endochondral) or the bones are formed directly from specialized mesenchymal cells (membranous). Remodeling of bone continues throughout life. Bone formation is regulated by osteoblasts and osteocytes and is defined by the synthesis of bone matrix and subsequent incorporation of bone mineral. This has been discussed with reference to the structure and the biochemistry of the bone (p. 856).

Bone Resorption. Bone resorption is controlled partly by osteoclasts and partly by osteocytes. They liberate substances which dissolve the bone matrix, releasing calcium and phosphate. Thus, the product of calcium × phosphate in the plasma is elevated. The calcium arises either from old bones which are destroyed by cellular resorption, or from the exchangeable calcium pool, in particular from the osteons which are not fully mineralized. When the calcium × phosphate product in the plasma falls, calcium is immediately released from the exchangeable portion of the bones (ROSENBAUM, 1964). Bone resorption begins only later, when apatite crystals are dissolved by osteoclasts and osteocytes under the influence of parathyroid hormone, and calcium is released predominantly from the old, nonexchangeable parts of the bone.

The rapid incorporation and release of calcium from the bones may be regulated by the *osteocytes*. The processes of the osteocytes are separated from the bone by a layer of amorphous calcium phosphate (BAUD, 1968). A halo around the osteocytes indicates an increased bone resorption and increased release of calcium (BÉLANGER, 1969) (p. 856). The multinuclear *osteoclasts* are produced only within hours to days under the influence of parathyroid hormone and cause dissolution of bone mineral. This process was described by KOLLIKER as early as 1873. Acid phosphatase is the predominant histochemically demonstrable enzyme in osteoclasts. It can be elevated in the plasma in hyperparathyroidism. The question as to whether the osteoclasts are principally phagocytic cells or whether they initiate and regulate bone dissolution has been asked over the years. KOLLIKER'S assumption that the formation of the osteoclasts precedes bone dissolution has been confirmed histologically, by electron microscopy (HANCOX, 1963) and in cultures of bone cells *in vitro* (GAILLARD, 1961). Osteoclasts predominantly dissolve the bone and phagocytize the bone minerals only temporarily. This has been demonstrated in autoradiographic experiments with plutonium, which can be observed first on the bone surfaces and later in the cytoplasm of the osteoclasts (ARNOLD, 1957). The osteoclasts have a brush-border and numerous vacuoles and vesicles which are demonstrable by electron microscopy (GONZALES, 1961). The dark grains in the mitochondria are presumably calcium phosphate crystals (PEACHEY, 1964).

Dissolution of apatite occurs when there is a fall in pH, formation of complexes with calcium or exchange of calcium ions with cations such as sodium. The local pH would have to fall below 6.8 for dissolution of bone minerals to occur, but no such fall has been demonstrated. The calcium balance becomes definitively more negative when the pH of the arterial blood falls, but it has not been confirmed whether increased bone destruction and/or increased calcium excretion through the kidneys is the primary cause of the negative calcium balance (LEMANN, 1966; REIDENBERG, 1966). Citrate, the best chelator of calcium, occurs

only in insufficient concentrations in plasma and bones. There is still no explanation or evidence of an ionic exchange which would have a significant effect on the disintegration of the bone surfaces.

It is possible that pyrophosphate influences bone resorption. In addition to bone formation, it may also inhibit the dissolution of apatite crystals. Since pyrophosphate is normally present in the bones, this property could be of importance *in vivo*. According to this view, the pyrophosphate covering the bone crystal must be destroyed during bone resorption (FLEISCH, 1966b, 1968) (p. 889).

In analogy to the dissolution of the bone minerals, the bone matrix could also be dissolved by acids or by ionic exchange. Results are inconclusive. It is more likely that the bone matrix is dissolved by proteases formed in the osteoclasts or osteocytes. Parathyroid hormone promotes the enzymatic breakdown of collagen, although it has not been demonstrated whether this is a primary site of action (VAES, 1967). Bone resorption is enhanced particularly by parathyroid hormone, thyroxine, and vitamins A and D. It is inhibited by calcitonin.

c) Methods

Bone formation and resorption can be estimated histologically (morphometrically) and radiologically. Determination of calcium, phosphate, citrate, and alkaline and acid phosphatase concentrations in the plasma and of the urinary hydroxyproline excretion can be useful in assessment of bone formation and resorption, as can physical and chemical analyses of the bones and kinetic analysis based on the use of isotopes or stable strontium. Some methods described in this chapter are still at the experimental stage, but they have contributed considerably to the understanding of the pathogenesis of metabolic bone disease.

α) Morphometry of the Bones

Bone formation and resorption can be measured histologically at autopsy, or by means of biopsies from the iliac crest or from a rib. The biopsy findings have been found to agree with the postmortem findings. Withdrawal of a bone cylinder from the iliac crest is simple and the procedure can be performed in outpatients. It is suitable for assessing the course of a metabolic bone disorder since it can be repeated several times. A suitable set of instruments has been developed by BURCKHARDT (1966). An adequate number of normal control cases from all age groups is obviously necessary for the exact morphometric assessment of bone biopsies.

A massive increase of unmineralized osteoid is even visible in decalcified bone sections. However, undecalcified sections must be examined by a quantitative morphometric method for an accurate assessment of bone biopsies (FROST, 1963; MERZ and SCHENK, 1970a; JOWSEY, 1966).

The formation of new bone is evaluated either microradiographically or with the aid of tetracycline labeling. The two methods give similar results. Microradiography is based on the fact that radioopaque (calcified bone) can be distinguished from radiolucent (osteoid) regions

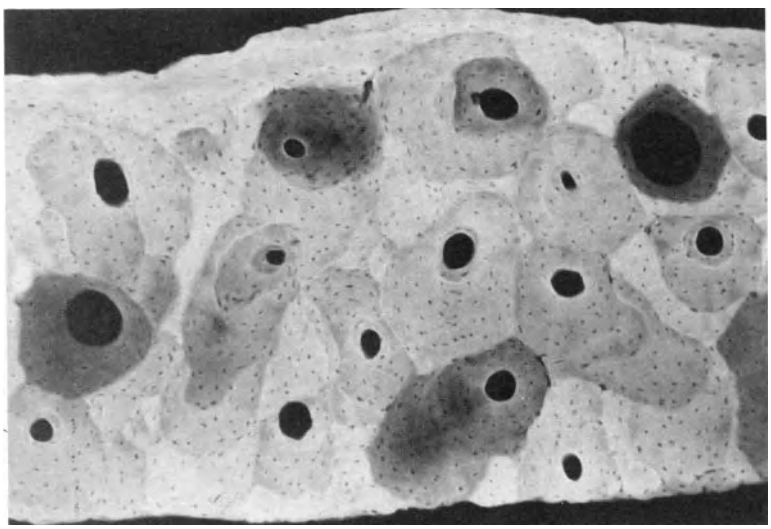


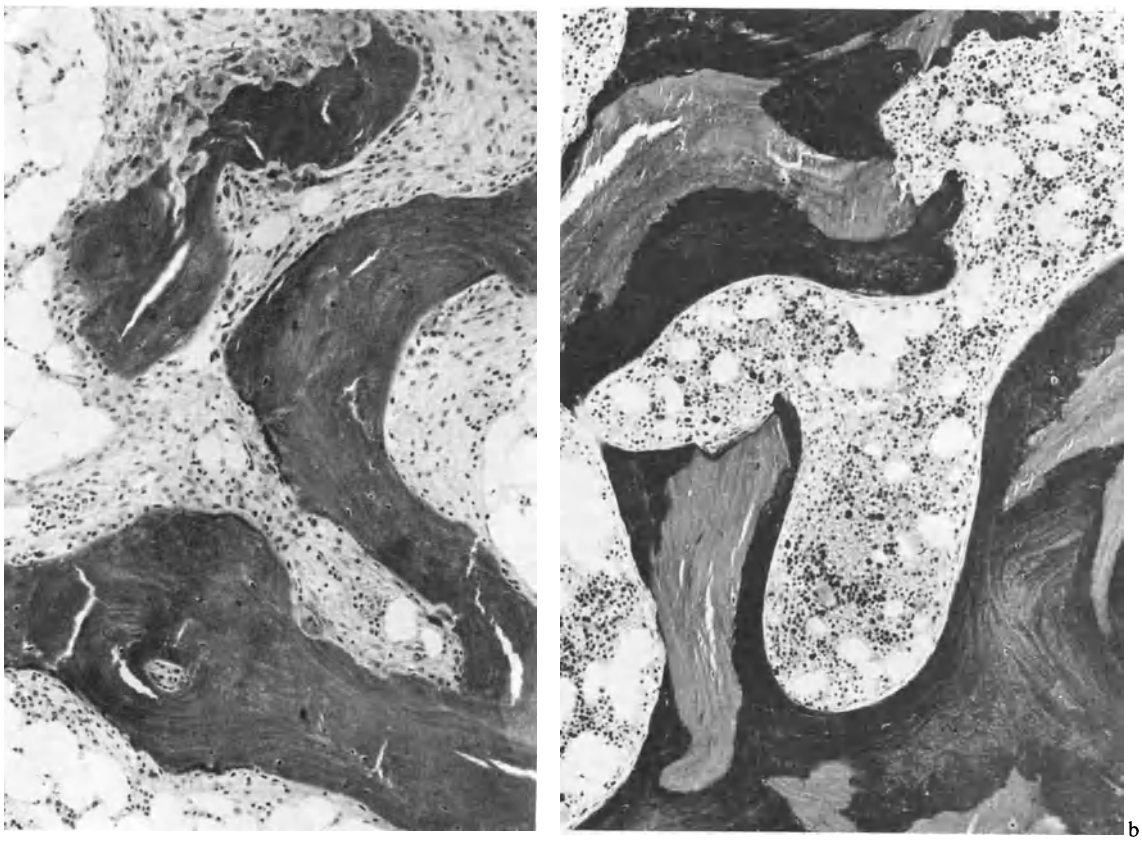
Fig. 5. Microradiograph of an undecalcified section of rib cortical bone. 80 : 1. The less radioopaque, darker parts are young, partly mineralized bones and the light areas represent the old parts of the skeleton. (Kindly supplied by Professor ROBERT SCHENK)

on a microradiophotograph of a bone section (JOWSEY, 1966) (Fig. 5). The microradiophotograph gives a measure of the size of the surface covered with osteoid. It reveals little about whether an increase of the osteoid is due to a rise of the formation of new bone or to decreased mineralization of the skeleton.

Labeling of the skeleton with tetracycline allows better differentiation between the two processes. Tetracyclines are only incorporated into metabolically active bone at the transitional zones from osteoid to apatite; from there tetracyclines are transported into the calcified bones (FROST, 1963; MÜLLER and SCHENK, 1966). The patient is given 1 g tetracycline per day (4 × 250 mg) for 4 to 6 days and if possible 10–30 days before the bone biopsy. The shift of the tetracycline from the surface of the bone into the calcified bone can be measured in a bone biopsy from a rib, and the volume of newly formed bone can be calculated as the newly formed volume per volume bone per year.

With the aid of tetracycline, active osteoid seams labeled with tetracycline can be differentiated from inactive, unlabeled areas in a biopsy taken from the iliac crest. The number of osteoblasts per osteoid surface can also be of aid in the assessment of new bone formation. If the number is increased relative to the amount of osteoid present, bone formation is increased, whereas in the absence of osteoblasts bone cannot be mineralized (MERZ, 1970a). This can be particularly well demonstrated in patients with osteomalacia due to intestinal malabsorption, in whom the number of osteoblasts is inadequate before treatment with vitamin D, causing a defect in mineralization. Treatment with vitamin D leads to increased numbers of osteoblasts and to subsequent deposition of bone mineral (FISCHER, 1970).

Bone resorption is assessed by measuring resorption surfaces. Furthermore, Howship's lacunae with osteoclasts are distinguished from those without osteoclasts. The osteoclast index (the number of osteoclasts per 100 mm^2 trabecular bone surface in the iliac crest) gives so far the best estimate of osteoclastic resorption. The osteoclast index according to SCHENK (1969) is 2.6 $\pm$ 1.1 in over 100 random autopsies on controls between 20 and 80 years of age.



a b

Fig. 6a and b. Undecalcified sections of bone biopsies from the iliac crest, 5 µm, Goldner stain, 110 : 1. a) Primary hyperparathyroidism with fibroosteoclastic bone resorption (female, 52 years). b) Osteomalacia in intestinal malabsorption (idiopathic sprue) (male, 57 years), the red-stained osteoid, which is present in large amounts, appears dark. (Kindly supplied by Professor ROBERT SCHENK)

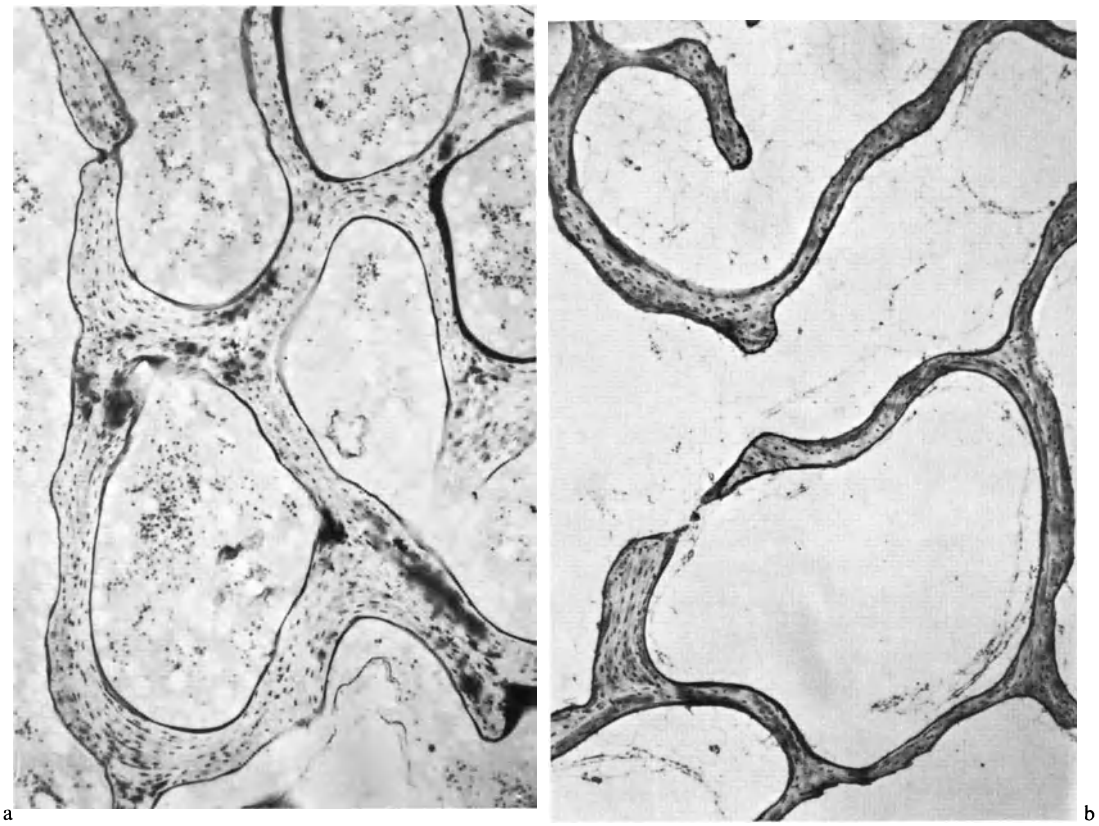


Fig. 7a and b. Undecalcified sections of bone biopsies from the iliac crest. Basic Fuchsin stain 45 : 1. a) Normal trabecular bone (male, 43 years). b) Osteoporosis with a decreased total bone mass (male, 75 years). (Kindly supplied by Professor ROBERT SCHENK)

The osteoclast index can be elevated or normal in hyperparathyroidism. Fibro-osteoclasia is qualitatively visible in about 10–20% of the cases with primary hyperparathyroidism (Fig. 6). As a rule, these patients also have radiologically visible subperiostal resorption zones in the phalanges of the fingers (p. 911). There are also surgically verified cases of primary hyperparathyroidism with a normal number of osteoclasts, in whom calcitonin may inhibit bone resorption (BINSWANGER, 1968a).

The volumetric *density* of the spongiosa is measured as the percentage of the bone trabeculae in a histological section. Regardless of the rate of bone formation or resorption *osteoporosis* is defined as a loss and *osteopetrosis* as an increase in volumetric density (FROST, 1963; MERZ, 1970b) (Fig. 7). Osteoporosis can be caused by decreased bone formation or by increased bone breakdown.

Hyperthyroidism and Cushing's disease have been chosen as examples, since the value of the morphometric assessment of the histology of the bones is demonstrated in these two disorders. A loss of bone substance, i.e. osteoporosis, is common to both. Hyperthyroidism causes more rapid remodeling of the skeleton with increased bone formation. The osteoid mass is sometimes increased. In contrast to the mineralization defect in rickets or in osteomalacia the major part of the osteoid tissue stores tetracycline and is covered by osteoblasts. It is therefore metabolically active. The bone resorption is also increased and exceeds the formation of new bone. The end result is osteoporosis and a loss of bone mass. In Cushing's disease or cortisone medication, however, bone remodeling is slowed down. Bone resorption is dominant in all these conditions. The end results are clinically and radiologically very similar, although the pathogenesis of the two forms of osteoporosis is fundamentally different.

β) Bone-Cell Cultures

The synthesis of the bone matrix and possible sites of action of hormones can be observed in in-vitro cultures of bone cells (PROCKOP, 1967). As a rule, embryonic bone splinters from the vault of the skull or from the metaphysis of the long bones are used; bone biopsies from

patients with hyperparathyroidism have also been compared with those of normal controls (FLANAGEN, 1965).

Triiodothyronine *in vitro* causes a promotion of the uptake of amino acids into embryonic cartilage cells (ADAMSON, 1967). Morphometric and kinetic results indicate that bone resorption is promoted in particular, and bone formation to a lesser extent. Undifferentiated mesenchymal cells can imitate the function of osteoblasts, osteocytes or osteoclasts. The results of metabolic investigations are therefore only partly applicable to the conditions *in vivo*.

γ) Kinetic Studies of Bones

In this section, kinetic investigations depict the measurement of the movement of isotopes and of strontium, which are primarily exchanged with and incorporated into bones (BAUER, 1961; AUBERT, 1960; EISENBERG, 1961; HEANEY, 1958, 1964; PROCKOP, 1967). The diagnostic value of the kinetic investigations is still limited. They are of value in understanding the pathophysiology of the skeleton, especially when used in combination with other methods.

Hydroxyproline labeled with ^{14}C is used to investigate the metabolism of collagen. The mineral exchange in the bones is measured with radioactive calcium and strontium and also with cold strontium, since it occurs only in traces in bones.

Collagen. PROCKOP (1967) has promoted investigations with ^{14}C-labeled proline. The increased excretion of hydroxyproline peptides in the urine is a reflection of an accelerated collagen metabolism. Proline is incorporated into collagen and subsequently hydroxylated into hydroxyproline in the osteoblasts. Kinetic methods using ^{14}C-labeled proline can show whether the raised excretion of hydroxyproline peptides into the urine is due to increased collagen synthesis, to a block in the conversion of the "soluble" collagen into the "insoluble" collagen fibrils, or to an increase in the breakdown of collagen or hydroxyproline into CO_2 and urea (PROCKOP, 1967) (Fig. 4). The major reason for increased urinary hydroxyproline excretion is an elevated collagen breakdown (soluble and insoluble collagen), e.g. during growth, in hyperthyroidism and in hyperparathyroidism. During growth and after the administration of growth hormone there is an additional increase in collagen synthesis which is responsible for the increased excretion of hydroxyproline into the urine. In lathyrism and in Marfan's syndrome this is due to a disturbance in the incorporation of hydroxyproline into the bone matrix with a retardation of the conversion of the "soluble" collagen into "insoluble" collagen. Cortisone reduces the excretion of hydroxyproline, mainly by inhibiting collagen synthesis. Additional histological and kinetic investigations with radioactive calcium permit localization of the cause of an elevated hydroxyproline excretion. In hyperthyroidism and in hyperparathyroidism, increased bone resorption is the dominant feature. The hydroxyproline excretion is reduced postoperatively in hyperparathyroidism, reflecting the decreased bone destruction, although the alkaline phosphatase rises at the same time with a compensatory increase of the formation of new bone (MACDONALD, 1965). Urinary hydroxyproline excretion correlates better with bone resorption as measured by isotopic kinetic methods than with bone accretion (KLEIN, 1964, p. 864). The clinical importance of the urinary excretion of hydroxyprolines is discussed on p. 942.

Bone Mineral. In most methods a single intravenous injection of radioactive calcium or strontium or stable strontium is given, and subsequently the loss in the plasma and the excretion in the urine and feces are measured (Fig. 8). Calcium and strontium behave similarly towards the skeleton, and the discussion is therefore limited to calcium. In about 48 hours, the calcium reaches a state of equilibrium with the extracellular fluid and with the pool of the rapidly exchangeable calcium (abbreviated calcium pool). At the same time, the substitution of the rapidly exchangeable radioactive calcium in the bones through stable calcium and its excretion in the urine, feces and sweat cause a steady fall in the concentration of the isotope in the plasma. The amounts excreted can be measured in the stools and urine and the difference from the amount injected gives an indication of the amount incorporated into the bones. Some of the exchange processes are related to the metabolically active, growing osteons and are irreversible during the investigation. At the same time, an exchange occurs at the bone surfaces. This has also been observed in isolated hydroxyapatite crystals *in vitro* (PAK, 1967). A portion of the incorporated calcium is again released by bone resorption, presumably after a few days to a few weeks. The *retention* is obtained by subtracting the amount of radioactive calcium excreted in the urine and stools from the amount administered. A more accurate measurement is achieved by using a whole-body counter. The results obtained with this method are somewhat lower (NORDIN, 1965), since the excretion in the sweat is not

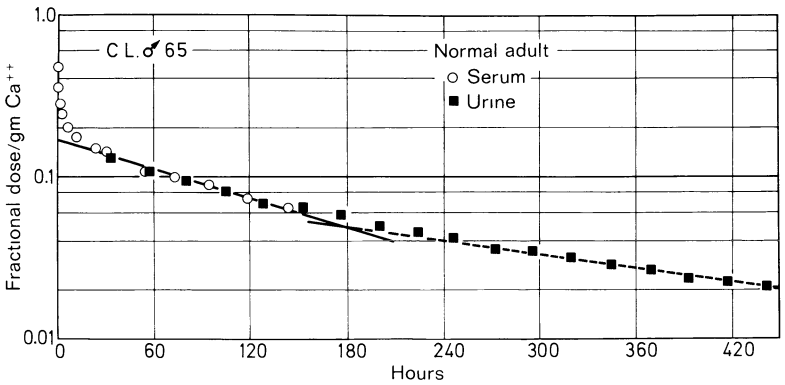


Fig. 8. Fractional dose of $^{45}Ca/g\ ^{40}Ca$ in the plasma and in the urine following a single intravenous injection of radioactive ^{45}Ca in a normal adult. (After HEANEY, 1958)

taken into account. The retention can be followed over weeks and months with a whole-body counter after one or several intravenous injections of radioactive ^{47}Ca or ^{85}Sr. The classic calcium balance (p. 850) gives similar information to the measurement of calcium retention. The curve obtained with a whole-body counter gives additional information about the rates of incorporation and removal of the calcium from the skeleton, insofar as the usual present-day models correspond to reality. The method is still in the experimental stage.

The calcium pool gives the theoretic distribution volume of calcium at the time of the injection. It is calculated from the ratio of the amount of isotope injected (Ri) to the theoretic plasma concentration at time zero (Rs_0). These quantities are obtained from the retrograde extrapolation of the fall in retention or the plasma concentration between the second and third and sixth and seventh days. The calcium pool is the sum of the extracellular distribution volume and the rapidly exchangeable calcium. The significance is controversial. The calcium pool is especially enlarged during growth. In rats, calcium administration has no influence on the size of the calcium pool (BRONNER, 1965). It is used in the estimation of bone turnover and accretion in the skeleton.

$$E = \frac{Ri}{Rs_0} \qquad (1)$$

E = calcium pool
Ri = amount of isotope injected
Rs_0 = concentration of the isotope at time zero
$T/2$ = half-life of the radioactive calcium in the plasma after intravenous injection, between the second and third and the sixth and seventh days
U = calcium turnover
Ca_U = excretion of calcium in the urine
Ca_F = excretion of calcium in the stools
R = resorption
B = calcium balance

The *turnover* (U) of the calcium between the calcium pool and the bones is calculated from the rate of fall of the calcium in the plasma or in the urine, multiplied by the calcium pool. $T/2$ is the half-life of the radioactive calcium in the plasma after the intravenous injection between the second and third days and the sixth and seventh days. 0.693 is an experimentally derived constant.

$$U = \frac{0.693}{T/2}\ E\,. \qquad (2)$$

The accretion or deposition (A) is calculated from the difference between the turnover and the excretion in the stools (C_F) and in the urine (Ca_U).

$$A = U - Ca_U - Ca_F\,. \qquad (3)$$

It is assumed that the metabolically regulated release of radioactive calcium occurs after 8–10 days at the earliest, as otherwise the accretion would be mainly a measurement of the retention of the calcium in the bones (p. 862). Turnover and accretion are increased during growth, in hyperthyroidism, in acromegaly and in Paget's disease. They are reduced in Cushing's disease. An increase in the accretion is paralleled by a rise in the concentration of the alkaline phosphatase in the plasma and indicates increased bone formation (KLEIN, 1964).

The *resorption* (R) can be calculated from the difference between the accretion and the calcium balance (B) (difference between calcium uptake and calcium excretion).

$$R = A - B\,. \qquad (4)$$

There is a good correlation between accretion and alkaline phosphatase, and between bone

resorption and the hydroxyproline excretion in the urine (KLEIN, 1964). Calcium administration inhibits bone resorption in rats, presumably by inhibiting the release of parathyroid hormone (BRONNER, 1965).

The kinetics measurements can be related up to a point to the formation of new bone, which can be demonstrated histologically. There is no strict relationship between accretion and the number of osteoid seams labeled with tetracycline or bone formation measured microradiographically (LEE, 1965; RIGGS, 1967). There is a better correlation in the young than in the adult dog, since during growth a large part of the calcium given is incorporated into the bones by metabolically active osteons, whereas in the adult the exchange between isotope and bone surfaces becomes a dominant feature. The results obtained in osteomalacia are contradictory. Histological examination after tetracycline labeling shows that bone formation is slow, whereas the turnover measured with strontium can be increased up to two-fold (FROST, 1963; FRASER, 1960). The osteoid borders labeled with tetracycline represent newly formed osteons. These osteons are autoradiographically visible as black spots ("hot spots"), in contrast to the diffusely distributed radioactive calcium (MARSHALL, 1959). The diffuse exchange processes cannot be determined histologically with tetracycline labeling. These processes may be important, particularly in adults, for the rapid regulation of the serum calcium concentration and for calcium homeostasis. The histological and kinetic investigations give additional and independent information. A great disadvantage of kinetic investigations is that the models used are crude simplifications and cannot be regarded as true reflections of pathologic conditions. A biopsy specimen is only a small part of the skeleton and may not be representative. Furthermore, purely morphological criteria are used, which do not allow measurement of the rates of bone formation or resorption.

δ) Physical Examinations

The breaking strength and the percentage deformation associated with fractures are dependent on the ash content per bone volume. This decreases with advancing age. However, the bone mineral remains qualitatively unchanged during adulthood (BELL, 1967).

ε) Radiographic Densitometry

In addition to the visual assessment of the form, structure and density of the skeleton, the bone density can be measured with the aid of strictly standardized conditions related to the intensity of the X-rays, geometry, and film properties. The density of the bone is measured at the vertebra in the hand and in the foot, and information can be obtained about the hydroxyapatite content of the bone. An aluminium hydroxyapatite scale with increasing density is laid alongside for comparison. The depth of the soft tissues is a hindrance in the assessment of bone density (KROKOWSKI, 1966). The measurement of the γ-absorption with ^{125}I gives superior results in thin bones (JOHNSTON, 1968).

The bone mass can also be estimated by measuring the depth of the compact bone in the bones of the hands, and by visual assessment of the bone density (GARN, 1967; BERNSTEIN, 1966). Structural alterations are discussed in the clinical section (see index).

ζ) Analytical Methods

Estimation of the ash content or the weight of the bone related to bone volume gives some indication of osteoporosis (ARNOLD, 1964; TROTTER, 1960). The calcium and phosphate contents can be referred to the wet or dry weight or to the nitrogen or nucleic acid content (p. 849). However, a disturbing factor is the absence of any point of reference. The nitrogen content gives a measure of the cell activity and of the size of the bone matrix. The nucleic acid content is related to the number of cells and the mitotic rate. The ash weight again takes no account of the bone matrix which is important for the assessment of the metabolic activity of the skeleton.

Radiological diffraction spectra and infrared absorption spectrophotometry allow investigation of the quality of the apatite. Bone minerals consist chiefly of hydroxyapatite and amorphous calcium phosphate. Traces of pyrophosphate are also present (POSNER, 1967).

4. Metabolic Bone Disease

Metabolic bone disorders indicate *generalized* involvement of the skeleton, in contrast to localized disorders due to a fracture or in osteomyelitis. Nevertheless, the involvement often only becomes clinically obvious in localized parts of the skeleton which are especially subject to strain and traction.

This section gives a general survey on alimentary and hormonal influences (except parathyroid hormone and calcitonin) and the effects of age and immobilization on the skeleton. Detailed descriptions are given in the appropriate chapters (see index).

a) Alimentary Influences

α) Calcium

A calcium-deficient diet can cause osteoporosis, particularly in growing and undernourished persons and in old age. An inadequate intake of calcium probably leads to an increased secretion of parathyroid hormone and a rise in bone resorption. In man, a calcium-deficient diet leads to increased intestinal calcium absorption, to an increase in bone resorption as measured by isotopic-kinetic analysis, and to the excretion of increased amounts of calcium and hydroxyproline in the urine. All these factors can be explained by an increased parathyroid hormone secretion rate (PHANG, 1968; SMITH, 1964). Conversely, in some cases an elevated intake of calcium leads to a positive calcium balance with a reduction of the isotopic-kinetically measured bone resorption, and possibly to inhibition of the release of parathyroid hormone (SCHWARTZ, 1965).

In cases with microradiographically visible increased bone resorption and decreased bone formation calcium infusions produce a normalization of these findings which may be due to inhibition of the parathyroid hormone secretion (PAK, 1969).

The density of the compact bone in the bones of the hands, measured radiologically in subjects receiving a calcium-deficient diet (< 300 mg/day) and others receiving a diet rich in calcium (> 1 500 mg/day), however, does not differ from that found in comparable population groups in Central and North American (GARN, 1967).

In adult cats, a calcium-deficient diet leads to osteoporosis which can be prevented by the administration of calcium or by parathyroidectomy (JOWSEY and RAISZ, 1968a). In rats, an elevated calcium intake causes inhibition of the isotopic-kinetically measured bone resorption, and possibly also of the release of parathyroid hormone, without any alteration of the serum calcium concentration (BRONNER, 1965). Intestinal calcium absorption is sometimes reduced in idiopathic osteoporosis during youth and old age (DENT, 1956; SPENCER, 1964). In these cases treatment with vitamin D and/or calcium leads to an improvement in the bone findings, without disclosing anything about the pathogenesis of the osteoporosis.

β) Vitamin D, Vitamin-D Resistance and Phosphate

Osteomalacia is characterized by an increase in unmineralized osteoid due to a defect in the mineralization of the bone matrix. Pathognomonic findings are pseudofractures and Looser's zones. Amorphous calcium phosphate is deposited into the osteoid, which is only slowly converted into apatite (POSNER, 1967). The osteoid mass can only be quantitatively assessed in undecalcified sections.

An increase of the osteoid mass alone is no proof of a vitamin-D deficiency. It can also occur in hyperthyroidism or in hyperphosphatasia, when it is a reflection of increased bone formation. The rate of collagen synthesis is normal or increased in both conditions. Osteoblasts and tetracycline deposits in osteoid seams are indications that bone formation is increased. Their absence suggests a mineralization defect such as occurs in vitamin-D deficiency (MÜLLER, 1966; BAYLINK, 1970; FISCHER, 1970).

Bone formation as measured by tetracycline labeling of osteoid seams, is retarded in osteomalacia (RAMSER, 1966). The fibro-osteoclasia is not always very extensive, especially in severe hypocalcemia, in intestinal malabsorption and in azotemia, since the permissive action of vitamin D on bone resorption is probably absent (RASMUSSEN, 1963; FISCHER, 1970).

The pathogenesis of the osteomalacia cannot be explained by the absence of a single factor, with the exception of vitamin-D deficiency in infantile rickets and of nutritive vitamin-D deficiency, which rarely occurs alone (DENT, 1969). The different forms of osteomalacia are therefore discussed separately.

Osteomalacia develops in vitamin-D deficiency in children (rickets), or as a result of resistance to the action of vitamin D (renal insufficiency, vitamin D-resistant rickets). It also arises in steatorrhea, after gastrectomy, in liver cirrhosis, with a phosphate-deficient diet or with a phosphate loss through the kidneys. Vitamin-D deficiency is the dominant feature in infantile *rickets*. At first the serum calcium concentration is usually only slightly reduced; the reduction later becomes severe, causing tetany. The serum phosphate concentration is low, and the phosphate excretion in the urine is elevated due to secondary hyperparathyroidism. The alkaline phosphatase in the plasma is raised before and immediately after treatment with vitamin D. It is a sign of increased osteoblastic activity.

Vitamin-D deficiency leads to osteoporosis in growing rats on a calcium-deficient diet. An increase of the osteoid mass only arises when the animals' diet is also deficient in phosphates (STEENBOCK, 1955). Parathyroid hormone deficiency alone, in contrast to vitamin-D deficiency, does not lead to a massive increase

in the osteoid mass. In the malabsorption syndrome with steatorrhea, osteoporosis sometimes precedes the osteomalacia, and can be treated successfully with vitamin D (GARNER, 1966; MUNCK, 1963; FISCHER, 1970).

Steatorrhea prevents normal intestinal absorption of the fat-soluble vitamin D, and furthermore, phosphate absorption is reduced, since a significant proportion of the phosphate is delivered to the organism in the form of fats. Finally, protein absorption is decreased, which is of importance in the synthesis of the bone matrix. In rare cases, there is no steatorrhea, and in one case only a reduction of the xylose absorption was demonstrable (MOSS, 1965).

Osteomalacia rarely occurs following *gastrectomy*. An elevation of the serum alkaline phosphatase may be related to an increased release of an intestinal phosphatase, especially from a blind loop. Osteomalacia was found in only 6 out of 1228 patients, and it was improved by treatment with vitamin D (MORGAN, 1965). In another series the vitamin-D concentration, measured biologically in the plasma, was reduced in 10 out of 28 patients and the osteoid seams widened at the same time (THOMPSON, 1966). In some patients, particularly the elderly, the bone density, measured radiologically in the metacarpal bone, is reduced (MORGAN, 1966). Mild, but frequent changes suggesting osteomalacia are reported by EDDY (1971).

In *renal insufficiency* the reduction of the calcium concentration in the plasma is the predominant feature at first. The osteoid surface is generally increased and tetracycline storage is limited to a few seams (BINSWANGER, 1968). When bone chips are incubated, calcium phosphate is deposited when certain concentrations of calcium and phosphate are reached. The calcification is impaired by the addition of plasma from uremic patients (YENDT, 1955). The acidosis occurring in uremic patients probably also contributes to the inhibition of the mineralization of the skeleton. Bicarbonate leads to a reduction of the calcium excretion in the stools and urine when serum calcium and phosphate are unaltered (LITZOW, 1967).

The intestinal absorption of calcium is reduced even when concentrations of vitamin D in the plasma are normal. This is corrected only with pharmacological doses of vitamin D. BELL (1964) observed a patient with sarcoidosis before and after the development of acute nephritis with azotemia: The calcium absorption, which was elevated at first, was reduced, while hypersensitivity to vitamin D was replaced by resistance. The hypocalcemia is not due to increased excretion of calcium in the urine. The calcium excretion through the kidneys is reduced, as is glomerular filtration. Resistance to vitamin D is thought to be the main cause of hypocalcemia (STANBURY, 1966).

The phosphate concentration in the plasma is elevated due to the reduced glomerular filtration rate. The hyperphosphatemia also leads to hypocalcemia and sustains the secondary hyperparathyroidism. Parathyroid hormone levels are elevated. They can be reduced with aluminium hydroxide gel or a phosphate-deficient diet (SLATOPOLSKY, 1970). During prolonged azotemia the osteomalacia disappears, but bone resorption increases and the serum calcium is normalized during stimulation of the parathyroid hormone secretion (STANBURY, 1966).

Osteomalacia connected with widening of the osteoid seams appears not only in vitamin-D deficiency, but also with a *phosphate-deficient diet* or with *phosphate loss through the kidneys*. In man, a massive increase of the osteoid tissue is produced in phosphate deficiency by the binding of the phosphate with aluminium hydroxide gels in the gut, and in rat by a phosphate-deficient diet (COLEMAN, 1950; LUDWIG, 1967; LOTZ, 1968). Vitamin D-resistant rickets results from a loss of phosphate through the kidneys. The calcium concentration in the plasma is normal. Calcium absorption from the gut and excretion through the kidneys are reduced. The phosphate loss is due to a defect in the renal tubules. The parathyroid hormone concentration is in the normal range (ARNAUD, 1971).

Treatment with vitamin D in doses which would normally lead to vitamin-D intoxication improves the mineralization of the skeleton. Calcium administration does not lead, as would be expected, to improvement of the osteomalacia. Calcium cannot be incorporated into the bones in the presence of a phosphate deficiency. Additional evidence that the rate of bone formation is slow comes from kinetic measurements, which show that the deposition rate is reduced, and from a decrease of the alkaline phosphatase in the plasma and the excretion of hydroxyproline in the urine (LAFFERTY, 1964; NAGENT DE DEUXCHAISNES, 1967).

Even treatment with *phosphate* alone improves the absorption of calcium and phosphate in the gut and the mineralization to some extent. This is associated with a rise in the calcium deposition, measured with ^{47}Ca. Furthermore, there is a decrease in the calcium concentration in the plasma and the calcium excretion in the urine, and an increase in the alkaline phosphatase level in the plasma and in the urinary hydroxyproline excretion, reflecting secondary hyperparathyroidism; the parathyroid hormone con-

centration in serum is increased following treatment with phosphate (NAGENT DE DEUXCHAISNES, 1967; ARNAUD, 1971).

γ) Fluoride

In North Dakota, subjects over 45 years of age who are exposed to drinking water with a high fluoride content have been found to have significantly higher bone density and less frequent compression fractures of the spine than comparable subjects living in South Dakota, where the fluoride content of the drinking water is lower. These findings are more common in women, although both sexes are subjected to the same environment (BERNSTEIN, 1968).

Fluoride can inhibit or stimulate bone resorption depending on the dose used (FACCINI, 1967; BAYLINK, 1970). In rats with immobilization osteoporosis, the calcium content of the bones is increased and the serum calcium reduced, which indicates that bone resorption has been reduced and formation of new bone is probably being promoted (GEDOLIO, 1966). Studies in growing rats, however, reveal an increase in periosteal bone formation, an inhibition of mineralization, and an increase in bone resorption (BAYLINK, 1970). In man, osteosclerosis with increased osteoid formation arises first, and after a latent period of a few months this is converted into apatite (JOWSEY, 1968 b). This delayed calcification is explained by an inhibition of the formation of bone matrix and of collagen synthesis by fluoride *in vitro* (PECK, 1965). The calcium balance becomes positive only after two to three months, and this is possibly related to the delayed calcification of the bone matrix (RICH, 1964).

A chronic fluoride intoxication leads to osteosclerosis and to increased fragility of the bones.

δ) Proteins

The *protein intake* influences the collagen synthesis and thus the extent of the bone matrix. Malabsorption causes osteoporosis as well as osteomalacia.

b) Age

Senile osteoporosis is the physiological loss of bone minerals with advancing age. Osteoporosis is characterized by a morphometrically measurable decrease in the volumetric bone density of the spongiosa, by a reduction of the thickness of the corticalis and by an enlargement of the Havers' compartments in the compacta (Fig. 7b). The radiologically measured thickness of the corticalis and the ash content or the bone weight per bone volume are decreased (ARNOLD, 1964; TROTTER, 1960). The thickness of the compact bone, measured radiologically, decreases slowly in the metacarpal bones from the beginning of the fifth decade. No definite connection with menarche and menopause in the woman is demonstrable, especially since the loss of minerals begins at about the same time, at the age of 40, in Central America (menopause at 40) and in North America (menopause at 50) (GARN, 1967). Senile osteoporosis is about four times more frequent in women than in men, (WHEDON, 1968). The mineral content of the bones at the end of the growth period is seldom known, and therefore little can be said, especially in individual cases, about the action of the menopause on the increased bone loss. Senile osteoporosis arises independently of the onset of the menopause and develops at the same time in the male and female. Senile osteoporosis cannot be discussed without mentioning alimentary osteoporosis, since this is implicated in the pathogenesis and in the treatment. Successful treatment discloses nothing about the pathogenesis of the senile osteoporosis. The same is applicable to the action of calcium, of estrogens and of androgens, which inhibit bone resorption in individual cases without causing any radiologically detectable improvement of the osteoporosis. As a rule, reduced bone formation can be demonstrated in senile osteoporosis. The number of newly formed osteons per year is reduced in these patients (VILLANUEVA, 1966). Two groups of patients with a reduced or compensatory increased number of osteoblasts and presumably formation of new bone become evident (MERZ, 1970). There may be some connection with immobilization osteoporosis, since muscular contraction could promote compensatory new bone formation in the presence of increased bone breakdown. In effect, histological morphometric examination of bone biopsies and kinetic studies using redioactive calcium reveal increased bone resorption as well as decreased formation of new bone (FROST, 1963; HEANEY, 1965). The findings in these bones presumably differ only quantitatively from those in hyperparathyroidism. Calcium deficiency promotes increased bone resorption due probably to increased parathyroid hormone secretion. The pathogenesis of senile osteoporosis is still largely unknown.

c) Immobilization and Gravity

In the absence of muscular contraction, bone formation is impaired and bone resorption

increased, leading to hypercalciuria, nephrolithiasis and occasionally in acute cases to increased serum calcium. The hydroxyproline excretion is higher during the night than during the day, reflecting increased bone breakdown at rest (MAUTALEN, 1970). The weights of the left psoas muscle and the third lumbar vertebral body are proportionally decreased or increased regardless of body weight and body length (DOYLE, 1970). Muscular contraction leads to an increase in the formation of new bone, possibly regulated through local piezo electric current changes at stressed bone surfaces (BASSETT, 1966). Movement is thus the best therapy for most forms of generalized osteoporosis. Increased bone resorption can lead to compensatory formation of new bone, a process which is best supported by movement and physiotherapy. An impressive example of immobilization osteoporosis is seen in astronauts in whom the action of gravity is also absent (LUTWAK, 1969). In rats, immobilization osteoporosis can be prevented by thyroidectomy or parathyroidectomy (BURCKHART, 1967).

d) Cortisone

Bone remodeling is retarded in Cushing's disease and following treatment with cortisone. The osteoporosis and the reduction in body length in children are caused primarily by a decrease in the formation of new bone. The osteoid mass is diminished and only small parts store tetracycline, indicating delayed mineralization of the skeleton. The excretion of hydroxyproline in the urine is decreased, reflecting the retarded collagen synthesis (HARTMANN, 1966). The uptake of radioactive ^{45}Ca by the bones *in vivo* is delayed (CLARK, 1959), as is the release of radioactive calcium from bone-cell cultures (STERN, 1969). In Addison's disease and after the removal of the adrenals in rats, the situation is reversed, and hypercalcemia with increased bone resorption arises, which can be prevented by thyroidectomy (JOWSEY, 1968c). *In vitro*, synthesis of polysaccharides and cartilage growth are retarded (BARRETT, 1966). The uptake of uridine labeled with ^{14}C of the ribonucleic acids, and the uptake of proline labeled with ^{14}C of the collagen are retarded in isolated bone cells by cortisone. It is probable that cortisone inhibits primarily the synthesis of collagen and of mucopolysaccharides, possibly at the site where the synthesis of ribonucleic acids is controlled by desoxyribonucleic acids.

Less bone matrix is formed when protein synthesis is inhibited, and osteoporosis then arises. It is not definitely known whether cortisone has an influence on bone resorption. The release of ^{45}Ca from the bones and collagen breakdown are not influenced *in vitro*. A reduction of bone resorption can be only poorly demonstrated in isotopic-kinetic and morphometric investigations. Hypercalcemic patients with multiple myeloma who are treated with prednisone show a reduction in the previously enlarged calcium pool, and in accretion and resorption rates (BENTZEL, 1964). The osteoporosis in Cushing's disease and following medication with adrenocortical steroids is due primarily to inhibition of the new bone formation with retarded synthesis of bone matrix. In addition to the retarded bone metabolism cortisone produces decreased intestinal calcium absorption and hypercalciuria, which could contribute to the negative calcium balance (KIMBERG, 1969).

e) Sex Hormones

Estrogens and androgens primarily inhibit bone resorption, and are therefore used for the treatment of senile osteoporosis. Castration causes a slight rise in the serum calcium and in the calcium excretion in the urine in man, which can be explained by increased bone breakdown (SZYMGNENDERA, 1967). The morphometrically measured bone formation and resorption, as well as the hydroxyproline excretion in the urine are reduced in the presence of estrogens (FROST, 1963; KATZ, 1968).

Isotopic-kinetic investigations and microradiographic findings in bone biopsies show that bone resorption is diminished by estrogens and androgens (GORDAN, 1963; RIGGS, 1969). The best possible result is a temporary increase in calcium and phosphate retention. Accretion is inhibited by a longer treatment. In addition, estrogens inhibit the growth of the epiphyseal cartilage and longitudinal growth of the bones in mouse, rat and rabbit (SIMMONS, 1966; BERNBER, 1968). The calcium balance remains uninfluenced (LAFFERTY, 1964; EISENBERG, 1966). ALBRIGHT'S theory (1948), which assumed from the findings of positive nitrogen balances following treatment with androgens that there was a promotion of bone formation, has not been confirmed.

The fact that estrogens and androgens produce a questionable inhibition of the increased bone resorption during the treatment of senile osteoporosis, says nothing about its pathogenesis. It is more likely that osteoporosis during the menopause is senile osteoporosis with a different course in the male and in the female (p. 867). The mode of action of estrogens and androgens is similar to that of the adrenocortical steroids. Bone resorption is retarded.

Estrogens and androgens primarily inhibit bone resorption and can retard the osteoporosis in some patients, whereas the adrenocortical steroids inhibit the formation of new bone and lead primarily to osteoporosis.

f) Thyroxine

Thyroxine causes an increase in bone remodeling. Bone formation and resorption are increased, the latter exceeding the former. The result is a reduction in the bone mass, i.e. osteoporosis. Bone formation is increased and this is occasionally reflected by an increase in the osteoid mass. In contrast to rickets and osteomalacia, the osteoid seams are narrow, they store tetracycline along the demarkation line and therefore do not show a mineralization defect. Mineralization of the skeleton is delayed in osteomalacia, whereas bone formation is increased in hyperthyroidism, as in the growth period and in acromegaly (FROST, 1963). The accretion, measured isotopic-kinetically, and the number of osteoclasts are sometimes increased in hyperthyroidism. They are usually below normal limits in hypothyroidism (BELL, 1967a; FROST, 1963). There is a good correlation between the hydroxyproline excretion in the urine and the concentration of protein-bound iodine in the plasma (KIVIRIKKO, 1965). The increased turnover of collagen becomes apparent in the skeleton and the skin, hence the thin skin of the patients with hyperthyroidism. The increased excretion of hydroxyproline and of calcium in the urine, and the increase in the osteoclasts as well as the hypercalcemia, which arises rarely, are indications of increased bone resorption. The possible inhibition of the release of parathyroid caused by the slight hypercalcemia could explain the inhibition of calcium absorption in the gut. Intestinal calcium absorption can also be inhibited by the rapid passage of the food through the small intestine. The inhibition of parathyroid hormone release also explains the hypercalciuria with an inhibition of the calcium reabsorption in the renal tubules. Hypercalciuria thus arises much more commonly than hypercalcemia in hyperthyroidism.

In rats, thyroidectomy prevents the development of immobilization osteoporosis (p. 867).

g) Growth Hormone

Growth hormone primarily promotes bone formation, with an increase in the mitotic rate of the osteoblasts. In cartilage-cell cultures, there is an increased incorporation of tritium-labeled thymidine into desoxyribonucleic acids under the influence of growth hormone after a latent period of 48 hours. Later, there is an increase in cell division (DAUGHADAY, 1966). The increased number of osteoblasts causes a rise in the formation of osteons in acromegaly (FROST, 1963). In acromegaly, bone resorption is sometimes increased, with a slight elevation of the serum calcium concentration leading to a negative calcium balance (BELL, 1967b; NADARAJAH, 1968).

h) Unexplained Causes

In addition to the decreased calcification of the bone matrix a high increase in the number of osteocytes with an increased bone breakdown can be observed in *osteogenesis imperfecta* (ROBICHON, 1968).

In *osteopetrosis* bone resorption is decreased and both the calcium absorption in the intestine and the calcium retention in the skeleton are increased. This could be caused by overproduction of calcitonin (p. 882).

In *idiopathic hypercalciuria*, accretion and bone turnover, as measured with radioactive calcium, are increased and the intestinal calcium excretion is relatively low compared with the excretion in the urine. The total calcium excretion i.e. into the urine and into the intestine, is not elevated. Increased bone remodeling could be the primary reason for idiopathic hypercalciuria (LIBERMANN, 1968).

5. Regulation of the Calcium and Phosphate Metabolism

Parathyroid hormone is the most single important regulator of the serum calcium concentration. The presence of vitamin D is necessary for parathyroid hormone to be able to exert its full effect on the intestine and the skeleton. Calcitonin causes a fall in the serum calcium concentration and serves as a rapid-onset counter-regulator. Skeletal growth and bone remodeling are further regulated by growth hormone, thyroxine, cortisol, and the sex hormones (see index, p. 868).

a) Parathyroid Hormone

α) Occurrence, Isolation, Secretion, and Metabolism of Parathyroid Hormone

Parathyroid hormone is formed in the parathyroids in all known animal species.

In addition to the highly purified parathyroid hormone (PTH), parathyroid extract (PTE, Lilly) is used in clinical medicine. PTE has a potency of about 100 units (USPU)/ml, or

10 units/mg dry weight. In contrast, the potency of highly purified parathyroid hormone is 2000–3000 units per mg dry weight. PTH was isolated in 1959 by counter-current distribution (RASMUSSEN and CRAIG; AURBACH). For later purification procedures gel filtration was extensively used (RASMUSSEN, 1964b; AUERBACH, 1967). Parathyroid hormone consists of a single polypeptide chain with a molecular weight of about 10000. The chain contains 84 amino acids. The total sequence of bovine PTH was derived in 1970 (BREWER; NIALL) (Fig. 9a). The first 34 amino-acid residues have been synthesized and the chain is still biologically active (POTTS, 1971a). The ability of bovine PTH to form antibodies remains intact after oxidation with a methionine or after treatment with cyanide bromide, while the biological activity is destroyed at the same time (POTTS, 1965).

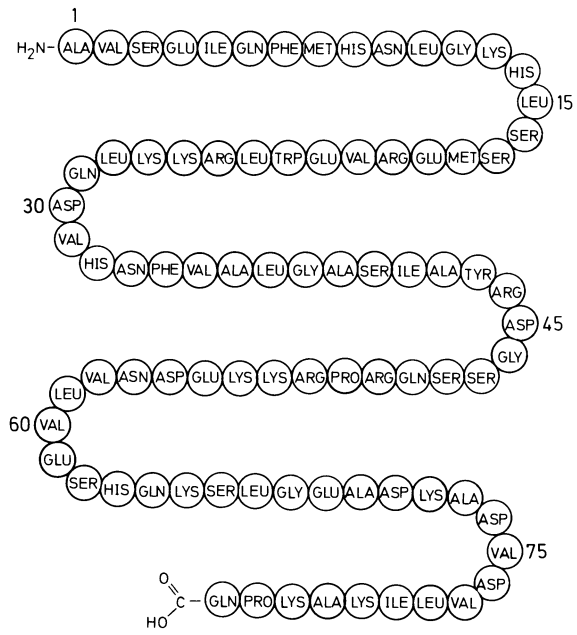


Fig. 9a. Amino-acid sequence of bovine parathyroid hormone (After BREWER, 1970; NIALL, 1970)

The sequence of the first 34 amino acid residues starting with the amino terminus of human PTH has been elucidated (BREWER and ARNAUD, 1972) (Fig. 9b). The synthesis has been accomplished and the chain is still biologically active (ANDREATTA, 1973). Antibodies have been raised against this peptide and the presence of immunoreactive PTH demonstrated in human serum using this system (FISCHER, 1974). Alternative structures for human PTH have been proposed by POTTS (1973).

Purified parathyroid hormone always leads to increased mobilization of calcium from the bones and thus to a rise of the serum calcium concentration of calcium. At the same time PTH promotes the excretion of phosphate through the kidneys and thus a fall in the serum phosphate concentration. The two actions cannot be separated. At least five peptides have been found in the parathyroids, and they show different biological effects, particularly *in vitro* (RASMUSSEN, 1964b).

The potency of parathyroid hormone is examined by its action on the mobilization of calcium from the skeleton (MUNSON, 1955) and by the phosphate excretion through the kidneys. The most frequently used assay is performed in the parathyroidectomized rat, in which the rise of the calcium concentration is measured after an injection of an extract of unknown potency. The increase in the serum calcium concentration and the phosphaturic action can also be tested in men. In order to prevent anaphylactic shock, which is theoretically possible, the extract must be given subcutaneously and applied on to the conjunctiva first. An intravenous injection of 200 units PTE does not normally cause any symptoms. One

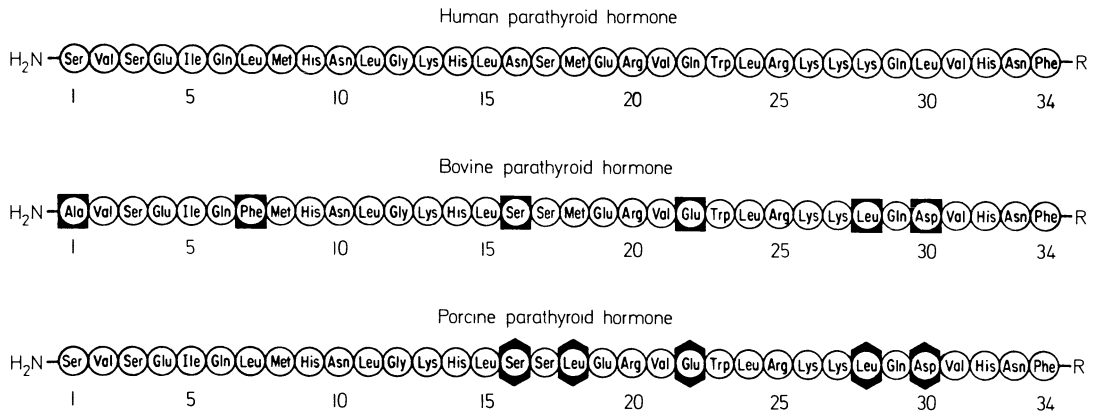


Fig. 9b. Comparison of the amino acid sequences of amino-acid residues 1–34 of human, bovine, and porcine parathyroid hormone. (After BREWER, 1972, 1970; O'RIORDAN, 1971)

thousand units PTE given over 12 hours in an infusion occasionally gives rise to shivering, fever and headaches. To our knowledge, severe complications have never occurred. Unpurified PTE is not used therapeutically as it leads to the formation of antibodies after a relatively short time. These antibodies have been demonstrated by radioimmunological methods (MELICK, 1967).

The concentration of the parathyroid hormone is measured in the plasma, urine and extracts of the parathyroids or of paraneoplastic tumors such as bronchial carcinoma and hypernephroma (GOLDBERG, 1964; SHERWOOD, 1967). Biological methods are sensitive enough for estimation of the hormone activity in organs and in the urine (ELICE, 1965). BERSON's radioimmunological methods are the most suitable for measurement of the concentration of PTH in serum (1966), and permit normal subjects to be differentiated from patients with hyperparathyroidism (REISS, 1968a; ARNAUD, 1971) (Fig. 10). At present bovine and porcine PTH are used as antigens. Their cross-reactivity with human PTH is not known quantitatively (BERSON, 1966; ARNAUD, 1971).

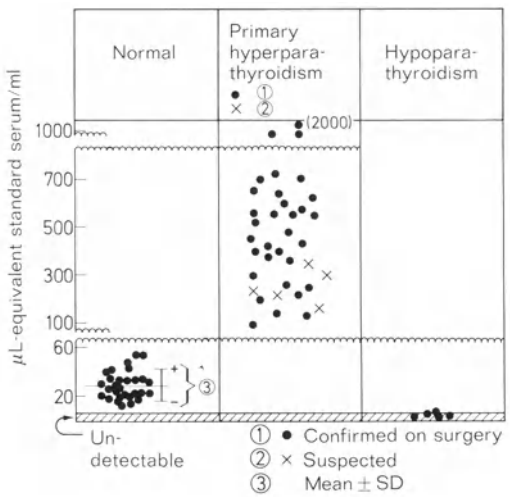


Fig. 10a. The concentration of parathyroid hormone in the serum of normal subjects and in patients with hypo- and hyperparathyroidism (After REISS, 1968a)

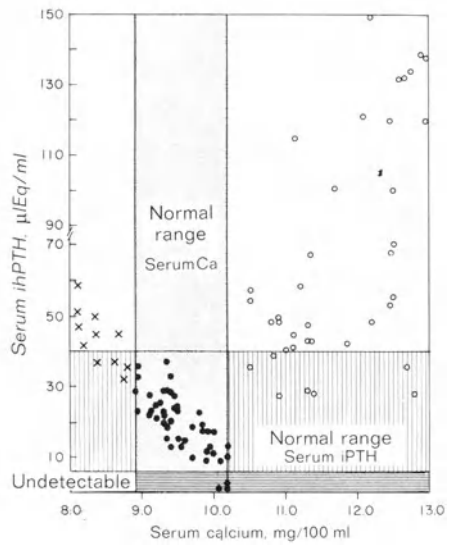


Fig. 10b. Relation between concentrations of serum calcium and immunoreactive parathyroid hormone (iPTH) in normals (●), in hypocalcemic patients (×) and in patients with surgically verified primary hyperparathyroidism (⊙). (After ARNAUD, 1971)

The level of PTH in serum can be estimated by indirect methods for clinical purposes. The assessment of hyperparathyroidism is based on the summation of the biological sites of action, and the single most important measurement is an accurate determination of the serum calcium concentration. The *parathyroid hormone concentration* in the plasma varies inversely to the serum calcium and magnesium concentrations. A fall in the serum calcium results in an increased secretion of parathyroid hormone (Fig. 11). It is the concentration of the ionic fraction rather than the total serum calcium concentration which regulates the PTH secretion. This has been clearly shown with phosphate infusions in the cow, where a lowering of the ionic calcium concentration preceded a decrease in total calcium. In this situation a rise in PTH concentration occurs within minutes of the fall in ionic calcium, while the total calcium concentration remains unchanged (FISCHER, 1973). Infusion of a parathyroid of the goat with blood rich in calcium or magnesium leads to inhibition of the secretion of parathyroid hormone (CARE, 1966). The quantity of parathyroid hormone which can be extracted from parathyroid glands is relatively small. If it can be assumed that most of the hormone is extracted by the usual procedures, this would suggest that the content of parathyroid hormone in the parathyroids is renewed 3 to 15 times per hour by synthesis in cows (SHERWOOD, 1966, 1968; BUCKLE, 1968). In normal control subjects the concentration of parathyroid hormone in the serum is inversely proportional to the calcium concentration in the serum. The serum calcium and PTH concentrations have to be taken into account in the assessment of borderline cases of primary hyperparathyroidism (ARNAUD, 1971).

The parathyroids are hyperplastic in rats fed on a calcium-deficient diet, although the serum calcium concentration may remain in the normal range. Acromegaly can be connected with hyperplasia of the parathyroids. Presumably the rise in the phosphate concentration

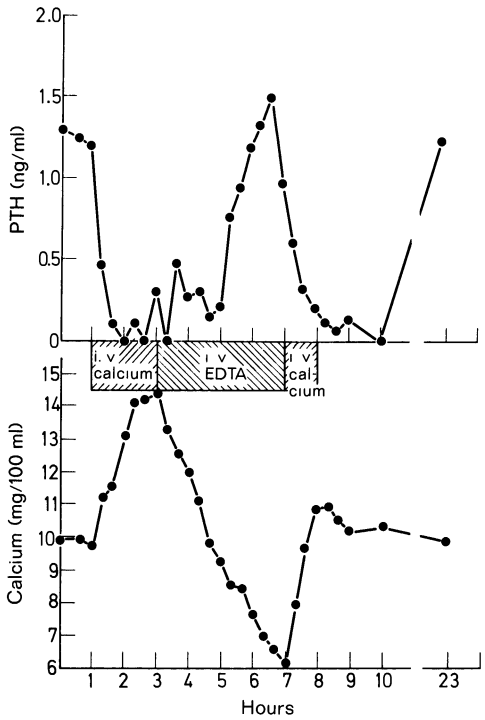


Fig. 11a. Concentrations of parathyroid hormone and calcium in bovine plasma in response to alternate infusions of calcium chloride (196.4 mEq/h) and disodium EDTA (115 mEq/h). (After SHERWOOD, 1966)

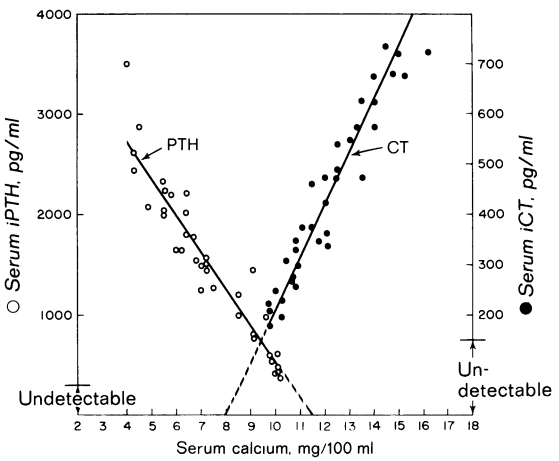


Fig. 11b. Relation between concentrations of serum calcium and immunoreactive parathyroid hormone (iPTH) and calcitonin (iCT) in pigs. r for iPTH = −0.942, for iCT = 0.964; p for both 0.001. (After ARNAUD, 1970)

in the plasma leads to frequent unmeasurable falls in the ionic calcium concentration, which cause secondary hyperparathyroidism. Rats on a high-calcium diet show decreased bone resorption, as measured isotopic-kinetically, with an unchanged serum calcium concentration (BRONNER, 1965). The parathyroid hormone concentration in the plasma in sarcoidosis is lowered in normocalcemic subjects, reflecting inhibition of the parathyroid hormone secretion with increased liberation of calcium from bone (REISS, 1968b; CUSHARD, 1972). A similar mechanism is assumed to be the cause of the normocalcemic secondary hyperparathyroidism with a decreased calcium absorption due to malabsorption. In addition to the hypocalcemia, frequent small unmeasurable falls in the serum calcium concentration are probably sufficient to act as a stimulus for an increased release of parathyroid hormone (FISCHER, 1970). Rats fed on a magnesium-free diet develop hyperparathyroidism with hypercalcemia, which can be prevented by parathyroidectomy (GITELMAN, 1968). A rise in the calcium and magnesium concentrations in the plasma leads to inhibition of the release of parathyroid hormone (CARE, 1966). A rise of the phosphate concentration in the plasma itself probably does not influence PTH secretion, except when it leads to a fall in the serum calcium concentration and subsequently to a rise in PTH secretion. The central nervous system and the pituitary have no demonstrable influence on the parathyroids. *In vitro*, a reduction in the calcium or magnesium concentration in the incubation medium leads to a rise in parathyroid hormone secretion and also to an increase in the uptake of amino acids and in the synthesis of nucleic acids and of proteins. An elevated calcium concentration leads to inhibition of these processes (RAISZ, 1969; SHERWOOD, 1970; OLDHAM, 1971).

Besides decreased serum calcium and magnesium concentrations, epinephrine is also potent stimulator of PTH secretion that might be of physiological importance (FISCHER, 1973).

The PTH is regulated by the serum calcium concentration in primary hyperparathyroidism. It is not autonomous. As shown by POTTS (1971b) and confirmed by BINSWANGER and FISCHER (1972), reduction of the serum calcium concentration produces an increase in PTH in the serum, whereas calcium infusions inhibit PTH secretion. In chronic hypocalcemia with normal kidney function (intestinal malabsorption, pseudohypoparathyroidism) the PTH is increased in relation to the decrease in serum calcium. The PTH concentrations are in the same order of magnitude as in primary hyperparathyroidism. However, in patients with azotemia and severe renal insufficiency the PTH concentrations are usually higher because the metabolism of PTH in the kidney is retarded (MELICK, 1969; ARNAUD, 1971).

The *metabolism* of PTH was not investigated until recently. Subsequent to BERSON's (1968)

discovery of the immunoheterogeneity of PTH in serum, ARNAUD (1970) has demonstrated that the PTH in peripheral serum and in tissue culture medium from parathyroid slices *in vitro* is immunologically different from glandular PTH, whose sequence is known in the bovine species (BREWER, 1970; NIALL, 1970). It appears at present that PTH is probably secreted in its glandular form *in vivo* and subsequently converted into lower-molecular-weight species, which are primarily found in peripheral serum and in tissue culture medium (ARNAUD, 1970; SHERWOOD, 1970; HABENER, 1971). The lower-molecular-weight forms are biologically active (FISCHER, 1972a; REISS, 1972). A converting enzyme has been extracted from parathyroid glands, liver and other tissues, which facilitates the conversion of PTH from its glandular form into the form which primarily occurs in serum. This conversion is stimulated in the presence of EDTA or EGTA, and the converting enzyme is a possible site of action for the regulation of PTH secretion by calcium. Furthermore, a calcium-binding protein has been isolated from parathyroid tissue with a high affinity (log K_f 5.5) for calcium. This is another possible site for the regulation of PTH secretion by calcium (OLDHAM, 1971b).

There is convining evidence that PTH circulating in the serum of patients suffering from paraneoplastic hyperparathyroidism, which has different immunological properties from the PTH circulating in patients with primary hyperparathyroidism, has a different molecular structure (RIGGS, 1971).

The metabolic *breakdown* of PTH occurs within hours in the plasma. The half-life of the bovine glandular hormone is 10–20 min in man, and 20–30 min in the cow, regardless of the serum calcium concentration (ARNAUD, 1967; SHERWOOD, 1968). MUNSON (1955) demonstrated hypocalcemia after two hours in parathyroidectomized rats on a calcium-deficient diet. DAVIS (1960) found that parathyroid transplants were more effective in the testes than in the spleen, and concluded therefore that the hormone was destroyed in the liver. These transplantation experiments exclude a nervous control over the liberation of parathyroid hormone from the parathyroids. ORIMO (1965) and MARTIN (1969) demonstrated that the breakdown of iodine-labeled parathyroid hormone is higher in the kidneys *in vitro* than in the spleen, skeletal muscle and liver. The half-life in the plasma is prolonged in patients with renal insufficiency or after nephrectomy (MELICK, 1969). Parathyroid hormone, which can be immunologically demonstrated, disappears from the plasma more slowly in uremic patients following parathyroidectomy than in hyperparathyroid patients (BERSON, 1968).

β) Sites of Action of Parathyroid Hormone

The most important sites of action are the bones, the kidneys and the small intestine.

Bones. Parathyroid hormone causes an initial fall followed by a rise in the calcium concentration in the plasma. Immediately after an injection of parathyroid hormone the serum calcium concentration decreases. This decrease is followed by a sustained rise. The initial decrease is explained by a sequestration of calcium phosphate in bone and in soft tissues. The initial decrease was usually attributed to calcitonin, until this effect was confirmed by PARSONS (1971) with homogeneous parathyroid hormone. The hypercalcemia is produced primarily by an increased mobilization of calcium from the bones and increased bone resorption, with an increase in osteoclastic and osteocytic activity. It is presumably the osteoclasts that are responsible for the long-term action of parathyroid hormone in bone remodeling, whereas the promotion of tubular calcium reabsorption by the kidneys, the calcium absorption in the gut, and the antagonistic action of parathyroid hormone and of calcitonin on the osteocytes are essential for the regulation of the serum calcium concentration (RASMUSSEN, 1967b). The significance of the action of parathyroid hormone on the osteocytes is further supported by the fact that the activity of osteoclasts can be suppressed by ^{239}Pu or by paraaminosalicylic acid, without significantly interfering with the mobilization of calcium from the bones, which occurs under the influence of parathyroid hormone (DOTY, 1965). Parathyroid hormone promotes the release of calcium from around the osteocytes which are then surrounded by a halo (BÉLANGER, 1968; FEINBLATT, 1970). The destruction of the bone matrix around the osteocytes by gelatinase can be demonstrated photographically on the surface of a film.

Parathyroid hormone causes an increase in the alkaline phosphatase in osteocytes (BÉLANGER, 1963). This can be demonstrated histochemically. Pyrophosphates protect bone crystals from dissolution *in vitro*. The alkaline phosphatase might be a pyrophosphatase, which would stimulate the metabolism of pyrophosphates and the dissolution of bone minerals under the influence of PTH (FLEISCH, 1970; RUSSEL, 1968) (p. 889).

The promotion of osteolysis via osteoclasts is one of the chief actions of parathyroid hor-

mone. Since BARNICOT (1948) observed that fibroosteoclasts were demonstrable after parathyroid tissue was transplanted over the bone *in situ* there has been no doubt that parathyroid hormone acts directly on bone, resulting in increased bone breakdown. In a similar manner, a direct action of parathyroid hormone has been demonstrated *in vitro* in bone cell cultures: there was an increase in the number of osteoclasts, and in the amounts of acid phosphatase released from osteoclasts. Prelabeled ^{45}Ca and hydroxyprolines were liberated, indicating the simultaneous dissolution of mineral and collagen (GAILLARD, 1961; RAISZ, 1965; SUSI, 1966). Calcium is primarily mobilized from the older parts of the bone. The increased release of calcium is only observed if the bones of the experimental animals have been labeled with radioactive ^{45}Ca three weeks prior to the administration of parathyroid hormone. When parathyroid hormone and ^{45}Ca are injected simultaneously, radioactive calcium is not mobilized from the newly formed parts of the skeleton (TALMAGE, 1965). A sudden fall in the serum calcium concentration leads to an immediate release of calcium from the bones, which is similar in parathyroidectomized rats and in normal controls. This sudden release of calcium from the bones is not regulated by parathyroid hormone (ROSENBAUM, 1964). A similar process also arises immediately after an injection of parathyroid hormone – the phosphate concentration in the plasma falls and there is an increase in the calcium released into the serum before the differentiation of osteoclasts or of osteocytes begins.

The action of parathyroid hormone on the bones consists predominantly in an increase in the mitotic rate, followed by an increase in the number of osteoclasts. An increased incorporation of thymidine into bone cells *in vitro* indicates a new synthesis of desoxyribonucleic acids and more frequent cell divisions (YOUNG, 1964). This effect is characteristic of parathyroid hormone and cannot be obtained in parathyroidectomized rats by means of peritoneal fluid rich in calcium. On the other hand, incorporation of ^{3}H-cytidine into ribonucleic acid can be stimulated by parathyroid hormone or calcium-rich peritoneal fluid (PARK, 1968). The increased release of calcium from bone cells cultures and the formation of osteoclasts is prevented *in vitro* by actinomycin-D. It inhibits protein synthesis at the level of the formation of messenger-nucleic acids (Fig. 9, Chap. I) (RAISZ, 1965 b; GAILLARD, 1965 a). Calcium mobilization from the bones, which occurs under the influence of parathyroid hormone, is also inhibited to a large extent *in vivo* in animals treated with actinomycin D (Fig. 12) (RASMUSSEN, 1964 a).

Experiments by TENENHOUSE (1966) provide further evidence of a cellular site of action for parathyroid hormone. PTH leads to an increased release of ^{45}Ca from bone chips incubated with ascites tumor cells. After centrifugation of the cells incubated with parathyroid hormone, a factor with different chemical properties from parathyroid hormone was isolated from the incubation fluid. This factor in itself leads to the release of calcium from the bone chips, whereas parathyroid hormone cannot exert its influence in the absence of cells. PTH stimulates the activity of an alkaline phosphatase in osteocytes. It is possible that this factor is the alkaline phosphatase, mentioned above, which as a pyrophosphatase accelerates the dissolution of the protective layer of pyrophosphate around the osteocytes, and thus promotes bone breakdown (p. 889). The connection is still hypothetical. Nevertheless, the fact that parathyroid hormone possesses a cellular receptor and that cells (osteoclasts or osteocytes) promote bone resorption, presumably through the release of enzymes, appears to be confirmed. VAES' results support this view (1967). He demonstrated an increased liberation of acid hydrolases such as beta-glucoronidase and of hyaluronidase from lysosomes which primarily dissolve mucopolysaccharides in bone-cell cultures under the influence of parathyroid hormone. The optimum pH for these hydrolases is an acid one. The concentration of the acid phosphatase is elevated in the plasma in a few cases of hyperparathyroidism and can be diminished with calcitonin *in vivo* and *in vitro* (DOTY, 1968; HEERSCHE, 1969). The synthesis of hydrolases precedes the action of parathyroid hormone. Actinomycin-D, which inhibits protein synthesis, also reduces the increased release of acid hydrolyses. This action is similar to the dissolving action of vitamin A on cartilage or to the phagocytosis of the leukocytes. In contrast to parathyroid hormone, the action of vitamin A can only be demonstrated in isolated lysosomes (FELL, 1962).

The release of proteolytic lysosomic enzymes through the action of vitamin A *in vitro* is inhibited by cortisone in a dose which has no influence on the increase of the number of the osteoclasts and the loss of bone minerals. Furthermore, calcitonin has no effect on the release of lysosomic enzymes *in vitro* despite its inhibitory action on calcium release and the formation of fibroosteoclasts. The increased release of hydrolases is therefore apparently not a direct effect of parathyroid hormone on lysosomes. The significance of these results

is also doubtful, since no collagenase has been demonstrated in lysosomes and collagen is the main constituent of the bone matrix (p.). A collagenolytic factor has been successfully demonstrated in bone-cell cultures, and this factor is activated by parathyroid hormone. The cellular site of origin of the factor is unknown (WALKER, 1964). Parathyroid hormone not only induces release of calcium from the bones, but also leads simultaneously to desintegration of the bone matrix. The increased excretion of *hydroxyproline* in the urine is an indication of the desintegration of the bone matrix. In hyperparathyroidism this rise is due to increased bone resorption rather than to raised collagen synthesis. The excretion of hydroxyproline decreases immediately after parathyroidectomy, although bone formation and the alkaline phosphatase in the plasma are increased postoperatively (MCDONALD, 1965). An infusion with calcium, which suppresses parathyroid hormone secretion and promotes the release of calcitonin, leads to inhibition of bone resorption as well as of the excretion of hydroxyproline in the urine (KEISER, 1964).

The dissolution of apatite into calcium and phosphate is promoted by the destruction of a protective layer of pyrophosphate, presumably under the influence of a pyrophosphatase (FLEISCH, 1966) (p. 889). NEUMANN (1958) further assumed that the dissolution of bone minerals could be promoted by an increase in hydrogen ions, as is found with the increased release of lactic acid under the influence of parathyroid hormone. Acetazolamide (Diamox), which inhibits carbonic anhydrase and thus also acidification, prevents a rise in the mobilization of calcium released by parathyroid hormone in the hen (SIEGMUND, 1965). A definite fall of pH to under 6.8 has not so far been demonstrated *in situ* at the bone surfaces.

Parathyroid hormone and vitamin D together lead to a rise in the citrate concentration in the plasma in the presence of an increased serum calcium concentration. Aconitase is inhibited by calcium, and the increase in citrate is possibly principally a result of the hypercalcemia. The hypothesis that the increase of citrate, and to a lesser degree of lactate dissolves the bones by acting as chelators of calcium is attractive but must be abandoned since the concentration of citrate in the bones is probably not even locally adequate for production of an increased calcium mobilization. A rise in the serum calcium concentration is also observed in rats treated with cortisone and under the influence of vitamin D without any simultaneous increase in citrate formation (HARRISON, 1957). An increase of citrate and lactate in the plasma in vivo and in bone-cell cultures in vitro merely indicates that increased amounts of glucose are metabolized with a liberation of lactate, citrate and CO_2. The increase of citrate is sometimes secondary to increased calcium uptake by osteoclasts with inhibition of aconitase activity and citrate breakdown (VAES, 1967; COHN, 1965). PTE inhibits isocitrate dehydrogenase *in vitro* in bone-cell cultures; the effects of calcium and/or parathyroid hormone cannot be differentiated (HEKKELMAN, 1963; HERRMANN-ERLEE, 1966). Increased oxygen consumption has been observed in isolated mitochondria incubated with parathyroid hormone and an oxidizable substrate (RASMUSSEN, 1966).

RIGGS (1965) demonstrated increased bone resorption in all the bone biopsies taken from patients with surgically verified primary hyperparathyroidism by microradiography. BINSWANGER (1968 a), in contrast, found an increased number of osteoclasts per trabecular surface in only half his cases of primary hyperparathyroidism which were confirmed by surgery. The number of osteoclasts remains constant throughout life in normal persons. It is justifiable to assume that in some patients with hyperparathyroidism with no increase in fibroosteoclasts, the counterregulation by calcitonin causes the disappearance of the osteoclasts, (p. 880).

New bone formation can be elevated or reduced in hyperparathyroidism. In some patients, increased bone resorption can lead to a compensatory increase in bone formation, as has been described in the case of immobilization osteoporosis (p. 867). WHITEHEAD (1959) found a reduced uptake of radiolabeled phosphate by the bones of rats treated with PTE. He deduced that bone formation was suppressed in this way. The compensatory new formation of bone does not occur in bone cell cultures *in vitro*, and an inhibitory action of parathyroid hormone on bone formation of bone appears probable.

The osteoblasts disappear from bone-cell cultures treated with parathyroid hormone within three to four days (GAILLARD, 1961). Morphological cell differentiation is difficult to observe in cell cultures. The inhibition of collagen synthesis and the uptake of proline and of glycine into the osteoblasts and into the bone matrix is a reflection of reduced osteoblastic activity (GAILLARD, 1965 b; YOUNG, 1964; OWEN, 1968; FLANAGAN, 1969). This effect cannot be explained by parathyroid hormone alone, since incubation in a calcium-rich medium can also inhibit collagen synthesis (PARK, 1968). Proline uptake into collagen is decreased in bone-cell cultures from patients with hyperparathyroidism with no radiologically

visible bone involvement and with a normal urinary hydroxyproline excretion. In cases with radiologically visible skeletal involvement and with an elevated hydroxyproline excretion in the urine, the proline uptake into collagen is increased, indicating increased compensatory new bone formation. The oxygen consumption is always raised (FLANAGAN, 1965). There is no definite demonstrable action of parathyroid hormone on bone formation.

Kidneys. In contrast to the action of parathyroid hormone on bone, which causes calcium to be mobilized within some hours, its action on the kidneys takes place within minutes. There is an increased excretion of phosphate which is independent of any alteration in the glomerular filtration rate. An injection of parathyroid hormone into a renal artery has the same effect (PULLMAN, 1960). In addition to the inhibition of phosphate reabsorption in the proximal tubules, the secretion from the distal tubules may also be increased since the phosphate clearance exceeds the creatinine clearance in some cases (WEBSTER, 1967). Selective destruction of the distal tubules by means of mercuric chloride leads to inhibition of the hormone-dependent excretion of phosphate. Stop-flow experiments in the dog indicate an inhibition of phosphate reabsorption in the proximal tubules (NICHOLSON, 1959; LAMBERT, 1963). There is still no unified concept as to whether parathyroid hormone has one or several sites of action in the kidney tubules.

Furthermore, reabsorption of calcium and of magnesium is increased under the influence of parathyroid hormone (RASMUSSEN, 1963a; MACINTYRE, 1963; NAMY, 1969). PTE causes an initial reduction of the excretion of calcium and magnesium in parathyroidectomized patients. After one to four days, this reduction gives way to an increase in calcium excretion (GILL, 1967). The hypercalciuria and the increased amounts of bicarbonate excreted at the same time, resulting in an alkalinization of the urine, sometimes lead to nephrocalcinosis and to the formation of kidney stones.

The increased tubular reabsorption of glucose which arises under the influence of parathyroid hormone has been used in some cases for the diagnostic investigation of hypercalcemia of unknown origin (HALVER, 1967). In addition, increased amounts of potassium, sodium, chloride, citrate, sulfate, bicarbonate, water and amino acids are excreted and more hydrogen ions and ammonia are reabsorbed. It is difficult to differentiate whether a disturbance is due to the primary action of parathyroid hormone or to the secondary tubular damage resulting from the hypercalcemia. This is especially applicable to the hyposthenuria due to hypercalcemia, which can arise from a disturbance of the counterflow system in the presence of a reduction of the sodium content of the renal medulla, or from vasopressin resistance in the distal tubules, or as the result of a direct action of parathyroid hormone on the renal tubules (EPSTEIN, 1959; FOURMAN, 1963).

Gut. Parathyroid hormone promotes the absorption of calcium, magnesium and phosphate by the intestinal mucosa. This effect can be demonstrated *in vivo* and *in vitro* in isolated intestinal villi following the injection of parathyroid hormone into the whole animal. PTH has no direct effect on indestinal villi *in vitro*. The uptake of ^{45}Ca is delayed in parathyroidectomized rats (RASMUSSEN, 1963; WINTER, 1970)

Ulcers develop in the stomach and the duodenum in about 10% of cases of hyperparathyroidism, regardless of the presence of islet cell adenomas of the pancreas, which occur frequently in familial polyglandular adenomatosis (ZOLLINGER, 1955; SCHMID, 1961). The ulcers usually regress after parathyroidectomy. Following administration of calcium, acid secretion of the stomach can rise before and after parathyroidectomy (BARRERAS, 1967; 1970). Calcium can cause increased liberation of gastrin in Zollinger-Ellison syndrome (TRUDEAU, 1969). A chronic increase of the serum calcium is not always associated with a rise in the gastric acid secretion (MURPHY, 1966).

γ) Mechanisms of Action of Parathyroid Hormone

The action of parathyroid hormone takes place in different phases at various sites of action. The increase of the phosphate excretion and of the calcium reabsorption in the renal tubules begins within minutes and is independent of any preceding protein synthesis. It also occurs in the absence of vitamin D. Presumably inhibition or promotion of a transport mechanism is involved. The elevated bone resorption which arises under the influence of parathyroid hormone, with increased release of calcium from the bones and raised hydroxyproline excretion in the urine, develops within hours and is preceded by an increase in protein synthesis, since bone resorption is raised only insignificantly in the presence of actinomycin-D (Fig. 12) (p. 874) (RASMUSSEN, 1964a). Vitamin D is essential to the action of parathyroid hormone on the bones (Fig. 13) (RASMUSSEN, 1963b). Calcium absorption from the small intestine takes an intermediary position. It

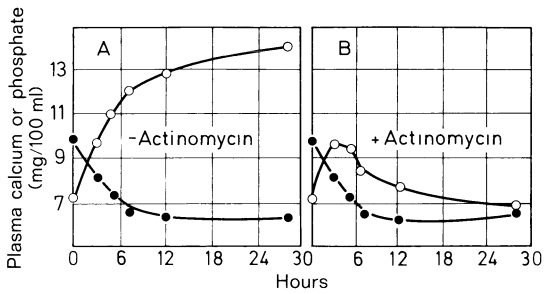


Fig. 12. The sequential alterations in plasma calcium (open circles) and phosphate (closed circles) after an intraperitoneal injection of 200 µg purified bovine parathyroid hormone into parathyroidectomized rats. Control values on left; values on right recorded in animals given 1 µg actinomycin-D/g body weight 2 h prior to injection of parathyroid hormone. (After RASMUSSEN, 1964)

comes into effect more slowly than the action on the kidneys and more rapidly than the calcium mobilization from the bones. On the other hand, the action on calcium absorption by the intestine is more quickly exhausted than the action on the bones (RASMUSSEN, 1961). The release of calcium from isolated intestinal villi is accelerated under the influence of parathyroid hormone only in the presence of vitamin D (RASMUSSEN, 1963a). PTE leads to increased synthesis of ribonucleic acids in the intestinal mucosa (HAMILTON, 1968).

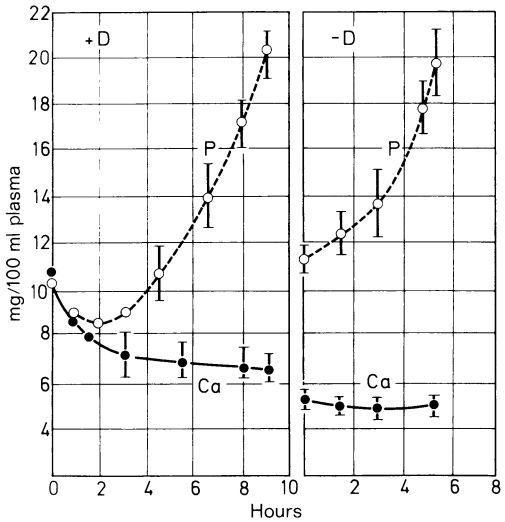


Fig. 13. Sequential changes in serum calcium and phosphate concentrations after parathyroidectomy by electrocautery in vitamin D-fed rats on a low-calcium diet (left) and of vitamin D-deficient rats on a normal-calcium diet (right). The decrease in serum phosphate 2 h after parathyroidectomy in vitamin D-fed rats (left) is significant ($p < 0.01$) compared with the initial value. The initial decrease in the serum phosphate in vitamin D-fed rats does not occur in thyroparathyroidectomized rats (in the absence of calcitonin) and the fall in the serum calcium is less steep. (Kindly supplied by Professor H. RASMUSSEN; after RASMUSSEN, 1963)

Increased administration of lactose leads to a rise of the calcium absorption by the intestine which is independent of parathyroid hormone and of vitamin D. Parathyroid hormone causes a rise in the serum calcium *in vivo*. This can be demonstrated even in the absence of vitamin D in animals treated with lactose and with calcium. *In vitro*, parathyroid hormone causes an increase in the calcium released from bone-cell cultures (AU, 1967).

Failure to respond to the administration of parathyroid hormone in cases with intestinal malabsorption does not definitely indicate vitamin-D deficiency or resistance of the skeleton to PTH, since the amounts of hormone administered can be too small to elicit a response in the presence of secondary hyperparathyroidism. The increase in osteoid mass found in hypocalcemia and in vitamin-D deficiency is at the most only a relative and not an absolute hindrance to the action of the osteoclasts on the skeleton.

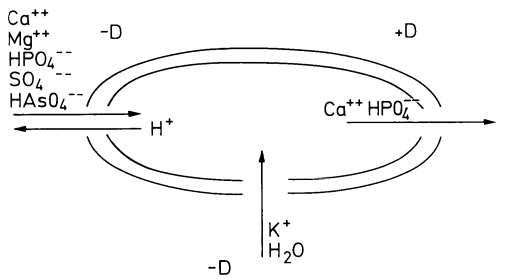


Fig. 14. Schematic representation of the action of parathyroid hormone on mitochondria *in vitro*. For some actions (+D) vitamin D is a requirement, others (−D) occur in the absence of vitamin D. For detailed explanations see text. (After RASMUSSEN, 1963, 1967)

The *mitochondrial membrane* is a subcellular site of action of parathyroid hormone. There is a close relation in the mitochondria between oxidative phosphorylation and ionic transport mechanisms under the influence of PTH. *In vivo*, the kidneys excrete increased amounts of phosphate, sulfate, potassium and water, and diminished amounts of hydrogen ions (Fig. 14). In mitochondria the uptake of phosphate, potassium, sulfate and water is promoted and the uptake of hydrogen ions suppressed (Table 2, Fig. 14). These effects can also be seen in the absence of vitamin D as they occur in the renal tubules. Some of the actions on the renal tubules are probably due to the hypercalcemia and to nephrocalcinosis, which cannot always be detected macroscopically. In spite of this, the analogy between the action on the renal tubules and on the mitochondria is impressive.

Table 2. Similarities between the actions of parathyroid hormone on the renal tubules and on mitochondria

	Excretion by the renal tubules in vivo		Uptake by the mitochondria *in vitro*	
	Stimulation	Inhibition	Stimulation	Inhibition
PO_4	+		+	
SO_4	+		+	
K	+		+	
H		+		+
Ca		+	+	+ (Release)
Mg		+	+	
H_2O	+		+	

The *magnesium concentration* probably holds a key position as an activator of the oxidative phosphorylation and of alkaline phosphatase or pyrophosphatase, which possibly accelerates the bone mineral dissolution around the osteocytes under the influence of parathyroid hormone. The uptake of magnesium into the mitochondria is increased under the influence of parathyroid hormone. Calcium is released from the mitochondria under the influence of parathyroid and/or of vitamin D. Like the calcium mobilization from bone, this release has only been demonstrated in mitochondria from rats previously treated with vitamin D (Fig. 15). These examples are meant to illustrate that mitochondria are suitable for the study of the mode of action of parathyroid hormone. A detailed description of possible sites of action of parathyroid hormone on the respiratory chain has been given by RASMUSSEN (1967a, 1966).

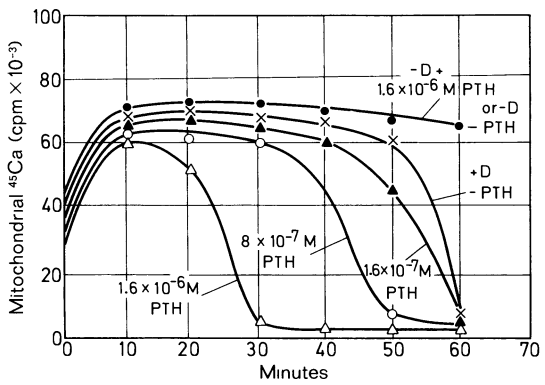


Fig. 15. The effect of parathyroid hormone and of vitamin D on the release of radioactive ^{45}Ca from mitochondria *in vitro*. (After DE LUCA, 1962)

A further possible site of action of parathyroid hormone is the *adenylcyclase*. After an injection of parathyroid hormone, the excretion of cyclic 3′,5′-adenosine monophosphate (cyclic 3′,5′-AMP) is increased prior to the onset of phosphate diuresis (CHASE, 1968) (Fig. 16). It has been demonstrated in labeling experiments that cyclic 3′,5′-AMP originates from the kidneys. A similar rise in the excretion of the cyclic 3′,5′-AMP and phosphate has also been achieved with high doses of vasopressin. Parathyroid hormone activates an adenylcyclase from the renal cortex *in vitro*; vasopressin, in contrast, an adenylcyclase from the renal medulla (CHASE, 1968). The activation of adenylcyclase is also promoted in bones under the influence of parathyroid hormone (CHASE, 1970). Most of the actions of parathyroid hormone can be imitated by an infusion of cyclic-dibutyryl-adenosine 3′,5′-AMP to the rat or of cyclic 3′,5′-AMP into the renal artery of the dog. The result is an increase in the serum calcium concentration

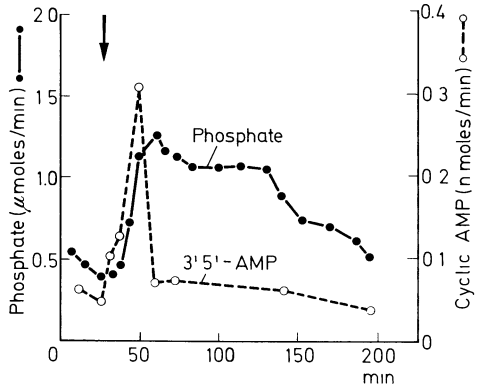


Fig. 16. Subsequent stimulation of urinary 3′5′-AMP and phosphate excretion by parathyroid hormone in vivo. (After POTTS, 1971)

and of the urinary hydroxyproline excretion and phosphate excretion (RASMUSSEN, 1968; RUSSELL, 1968b). There is suggestive evidence that cyclic adenosine monophosphate is the effector and second messenger of the action of parathyroid hormone. In patients with pseudohyperparathyroidism the parathyroid hormone concentration in the serum is increased; an infusion of parathyroid hormone leads neither to an increase of the renal phosphate excretion nor to an excretion of cyclic AMP; an adenylcyclase deficiency is postulated as the reason for the tubular resistance of parathyroid hormone (LEE, 1968; CHASE, 1969). Another explanation, however, is that in view of the raised endogenous serum parathyroid hormone concentrations, the amount of parathyroid hormone injected into these patients was insufficient to cause an increase in the urinary phosphate excretion.

In isolated renal tubules, parathyroid hormone stimulates the formation of 3′,5′-AMP and gluconeogenesis. In the absence of calcium in the incubation medium 3′,5′-AMP only is formed.

The effect on gluconeogenesis requires calcium (NAGATA, 1970). Parathyroid hormone promotes the calcium uptake in isolated renal cells (BORLE, 1970). The intracellular calcium concentration is crucial for the transmission of the parathyroid hormone action (RASMUSSEN, 1970).

In the kidneys and possibly in osteocytes the primary site of action of parathyroid hormone is probably a membrane which depends on metabolic processes (mitochondria, respiratory chain, adenylcyclase). In osteoclasts, the desoxyribonucleic acids of the cell nucleus probably play a major part in the control of the parathyroid hormone action and the synthesis of proteins or enzymes which stimulate the dissolution of bones. The exact mechanisms of action of parathyroid hormone remain to be elucidated.

b) Calcitonin (Thyrocalcitonin)

Calcitonin (CT) is a polypeptide hormone which causes the concentrations of calcium and phosphate in the serum to fall. The existence of a calcium-lowering principle, calcitonin (CT), was first suspected by COPP in 1961 (COPP, 1962). Calcitonin was then extracted by HIRSCH (1963) from the thyroid and named thyrocalcitonin. Thyrocalcitonin and calcitonin are identical. In most mammalian species including man CT is primarily a thyroid hormone. As a calcium-lowering hormone CT is the most important counterregulator of parathyroid hormone.

α) Occurrence, Isolation, Secretion, and Metabolism of Calcitonin

CT originates from the parafollicular or C cells (PEARSE, 1966a). The C cells were probably mentioned for the first time by BABER in 1877, but until recently no physiological importance was attached to them. On immunofluorescent examination C cells selectively bind antibodies to CT (BUSSOLATI, 1967). They are histochemically similar to the islet cells of the adenohypophysis (PEARSE, 1966). C cells are found in all cervical organs, in the thyroid gland, in the ultimobranchial body, in the parathyroids, in the thymus, and free in the neck (CARVALHEIRA, 1968). In pigs, CT has been extracted from the adrenal medulla (KAPLAN, 1970). In shark (*squalus suckleyi*) and hen CT has been extracted from ultimobranchial bodies. A small amount is found in the thyroid gland in hens, whereas equal amounts have been isolated from the thyroid gland and the ultimobranchial body of pigeons. Separate perfusion of the thyroid and the parathyroid glands of goats with calcium-rich blood has shown that CT arises from the thyroid (FOSTER, 1964). With the exception of the anteater, which has a separate ultimobranchial body, calcitonin primarily arises from the thyroid gland in mammalian species (COPP, 1967; MACINTYRE, 1967). In man, CT was demonstrated in the thyroid as well as in the thymus and in the parathyroids (GALANTE, 1968). Isolation and synthesis of porcine CT give evidence of a single polypeptide chain of 32 amino acids (RITTEL, 1968; GUTTMANN, 1968). Isolation and synthesis of CT from a medullary thyroid carcinoma in man also revealed a single chain with 32 amino acids; the structure, however, is markedly different. There are eighteen amino-acid residues in which human CT is diffent from porcine CT (Fig. 17) (NEHER, 1968; SIEBER, 1968). The structure of CT from the normal human thyroid is not yet known. The potency of CT is biologically tested in the rat or in the mouse by measurement of the fall in the serum calcium concentration. Growing animals with increased bone remodeling are more sensitive to CT than adult animals; a diet rich in phosphate promotes the calcium lowering effect (HIRSCH, 1968). In the rat bioassay, human and porcine CT do not significantly differ. However, salmon CT is biologically 20 to 30 times more active in man and rat (KEUTMANN, 1970).

The CT concentration in the plasma was first measured in the rat bioassay (STURTRIDGE, 1968). The use of a radioimmunoassay allows more accurate and reproducible determination of the CT concentration in the serum (LEE, 1969; CLARK, 1969; ARNAUD, 1970). Because of the

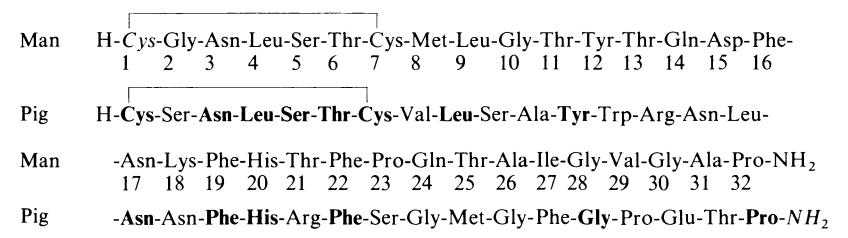


Fig. 17. Comparison of the amino-acid sequences of human calcitonin M (medullary carcinoma of the thyroid) and of porcine α-calcitonin. The amino-acid residues in heavy type are common to the human and porcine calcitonin. The first seven amino-acid residues are similar and the C terminals identical. (After NEHER, 1968)

different structures of human and porcine CT, which was exclusively used until 1968, the cross-reaction was, as expected, poor (TASHJIAN, 1969). CT from medullary carcinomas and synthetic human calcitonin M (SIEBER, 1968) were used for the generation of high-affinity antibodies to human CT (CLARK, 1969; TASHJIAN, 1970).

The *regulation of the CT concentration* in the serum by the thyroid is similar to the regulation of the parathyroid hormone concentration. Calcium and magnesium stimulate the secretion of CT directly, without the interposition of a feedback loop such as the pituitary or the central nervous system. A rise in the serum calcium leads to an increase in the CT concentration in the venous effluent of the thyroid (FOSTER, 1964; CARE, 1967). The CT concentration in the serum is proportional to the calcium concentration (LEE, 1968; ARNAUD, 1970). Isolated perfusion of the parathyroids with EDTA, which binds calcium, in contrast, leads to a rise of the parathyroid hormone concentration in the venous effluent from the parathyroids (CARE, 1966; ARNAUD, 1970).

Glucagon and cyclic adenosine monophosphate increase the secretion of calcitonin *in vivo* and *in vitro* (AVIOLI, 1969; CARE, 1969; BELL, 1970). The half-life of CT in the serum is similar to that of parathyroid hormone, lying between 4 and 12 min (LEE, 1969). The half-life of salmon CT in the circulation of the dog is much longer than the half-life of porcine CT, which explains the unusual potency of salmon CT in man (HABENER, 1971). The CT concentration in the thyroid is increased in chronic hypocalcemia. The parafollicular cells of the dog show an enlargement of the nuclei, an increase of intracellular vacuoles and a change in enzyme concentrations which can be histochemically demonstrated (PEARSE, 1966; HACHMEISTER, 1967; CAMERON, 1968). The CT content of the thyroid is slightly reduced in chronic hypercalcemia. Hypocalcemia probably causes an inhibition of the release of CT from the thyroid and the storage of secretory granules containing CT in the thyroid gland of rat (GITTES, 1968).

There is no fall in the CT concentration in the plasma of thyroidectomized patients, as measured in the rat bioassay, since CT is also synthesized in other organs (GUDMUNDSON, 1969; KAPLAN, 1970). The thyroid protects rats from hypercalcaemia and nephrocalcinosis resulting from lethal amounts of parathyroid hormone (RASMUSSEN, 1967). Oral administration of calcium causes a temporary calcium increase only in thyroidectomized rats (GRAY, 1969). Parathyroidectomized rats have lower serum calcium concentrations than rats in which both the thyroid and the parathyroids have been removed. These results in rats provide evidence that CT in the serum is effective as a regulator of the normal serum calcium concentration (TALMAGE, 1965).

In contrast to the situation in parathyroidectomized rats, a perfusion of CT over 6 hours in intact rats leads to an increase of the fibroosteoclasts, presumably due to secondary hyperparathyroidism in the presence of hypocalcemia (EVANSON, 1967). The same situation can be produced *in vitro* in bone-cell cultures; parathyroid hormone and CT first inhibit and later even stimulate the release of calcium (FRIEDMAN, 1968).

In contrast to parathyroid hormone, the onset of the action of CT is rapid, but also quickly exhausted even when more CT is infused. Presumably CT is necessary for the regulation of rapid alterations in the serum calcium concentration, whereas parathyroid hormone is constantly released to maintain a normal calcium concentration in the serum.

Parathyroidectomy leads to a reduction in the serum calcium, whereas the removal of the thyroid, the major CT-producing organ in man, is not followed by hypercalcemia. Growth hormone promotes the calcium-decreasing action of CT. CT is more effective in growing rats. Hypophysectomy inhibits the action of CT (COPP, 1968). If bone remodeling, and in particular resorption, is increased, CT has a greater hypocalcemic effect. MILHAUD (1965) noted that the uptake of 131-iodine in the thyroid was barely measurable two weeks after hypophysectomy, whereas the CT concentration remained unchanged.

β) Sites of Action of Calcitonin

Bones: The most important action is the inhibition of bone resorption. In parathyroidectomized rats fed on a calcium-rich diet, CT causes a reduction in the number of fibroosteoclasts. The bone trabeculae become thicker, and the changes are accompanied by a corresponding increase in bone density on X-ray examination (FOSTER, 1966a). The loss of strontium and of hydroxyproline from the skeleton and the urinary excretion of hydroxyproline are reduced in rats treated with CT *in vivo* (KLEIN, 1968; KAHLER, 1966; MARTIN, 1966; RASMUSSEN, 1967). Perfusion of the isolated tibia with CT leads to an increase in the calcium retention in the bones (PARSONS, 1968).

In-vitro inhibition of the bone resorption was demonstrated in bone-cell cultures pretreated with parathyroid extract (ALIAPOULIOS, 1966; FRIEDMANN, 1968). Calcitonin and para-

thyroid hormone influence bone resorption via cellular sites of action. The inhibition of the release of ^{45}Ca from bone-cell cultures under the influence of CT or the stimulation by parathyroid hormone is lost after heat inactivation (RAISZ, 1967).

The formation of osteocytic lacunae under the influence of PTH in rats is lost if the animals are simultaneously perfused with CT (RASMUSSEN, 1967). The osteocytes are probably a site of action in the bone for CT and PTH (p. 856).

The dominant feature of CT is the inhibition of bone resorption. Stimulation or inhibition of bone formation has not been definitively found. Inhibition of bone resorption alone can result in increased retention of calcium in the bones, which can lead to hyperostosis. If bone resorption is suppressed calcium cannot be released into the plasma, and this leads to hypocalcemia.

Kidneys. CT increases the urinary excretion of phosphate even in hypoparathyroid patients without changing the glomerular filtration rate (HAAS, 1971; ARDAILLOU, 1967). The injection of CT in high concentrations into the renal artery of the dog does not lead to an increase of the phosphate excretion into the urine (CLARK, 1969). In contrast to parathyroid hormone, which leads to a prolonged rise in the phosphate excretion, CT only produces a temporary effect. The phosphaturic action may reflect a direct effect of CT on the kidney tubule or it may be caused by a lowering of the serum calcium concentration. The latter alternative is supported by the fact that EDTA, which lowers the serum calcium, produces an increase in phosphate excretion in parathyroidectomized rats (RASMUSSEN, 1967). The fall of the plasma phosphate and calcium can be demonstrated in nephrectomized and eviscerated animals, which shows that the major action of CT is the inhibition of bone resorption (GUDMUNDSON, 1966). CT does not produce a fall in the serum calcium in parathyroidectomized hypocalcemic rats, whose bone resorption is suppressed. The elevated serum phosphate does fall, however, and this can be prevented by previous nephrectomy (ROBINSON, 1967). These results could explain the changes in urinary phosphate excretion in patients with hyper- and hypocalcemia and varying concentrations of PTH and CT in the serum. They also offer an explanation for the changing phosphate excretion after the infusion of calcium in hyperparathyroid patients, and for the resulting uncertainties associated with the interpretation of the calcium infusion test (p. 943). In addition to the actions of CT on the bones and on the kidneys, CT also has other effects which are probably secondary to the lowering of the serum calcium concentration. These effects include the fall in the calcium concentration in the myocardium and in the kidneys (GUDMUNDSON, 1966; KENNY, 1965). A similar reduction of the intracellular calcium concentration has been described in hypocalcemia after parathyroidectomy, where, in contrast, there is a reduction of the CT concentration in the serum (WALLACH, 1966). The calcium absorption in isolated loops of the small intestine is not affected by CT (ROBINSON, 1968).

γ) Calcitonin in Man

The structure and synthesis of CT from medullary thyroid carcinomas are discussed on p. 879. The biological activity of human CT does not differ greatly from porcine CT in man. In the presence of increased bone resorption (Paget's disease and/or hypercalcemia) CT produces a rapid fall of the calcium and phosphate concentrations in the plasma (FOSTER, 1966c; HAAS, 1968). An intravenous injection of even 1 M.R.C. (Medical Research Council) unit of CT produces a significant calcium-reducing activity in the plasma of patients with hypercalcemia (FOSTER, 1966b) (Fig. 18). In normal control subjects calcium, magnesium, potassium, and phosphate excretion are increased in the absence of any lowering of the serum calcium concentration (SINGER, 1969). The CT concentration of normal human plasma can be measured by radioimmunoassay. It is elevated after an infusion of calcium in normal subjects and in patients with medullary carcinomas of the thyroid (STURTRIDGE, 1968; TASHJIAN, 1970). Thyroidectomy does not alter the bioassayable CT concentration in the serum, and the human

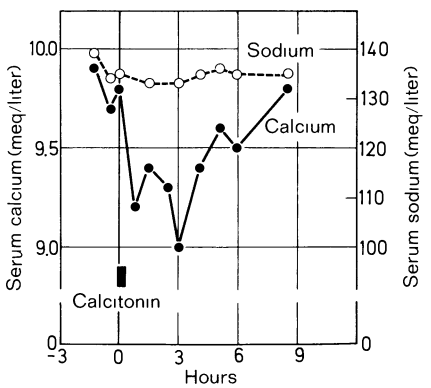


Fig. 18. Serum calcium and sodium concentrations after an intravenous injection of 1 MRC unit of porcine calcitonin in a patient with hypercalcemia resulting from skeletal metastases. (After FOSTER, 1966c)

thyroid is therefore thought not to be the only site of synthesis for CT (GUDMUNDSON, 1969). The clinical features of overproduction or deficiency of CT do not include any striking findings. Thyroidectomy does not lead to hypercalcemia in man. Deficiency of CT can be suspected in thyroidectomized patients if the half-life of calcium given by infusion proves to be 200 min, in contrast to 48 min in control subjects (WILLIAMS, 1966; MAZZUOLI, 1966).

An elevated concentration of CT in the thyroid and in the plasma has now been demonstrated in most cases with medullary carcinoma of the thyroid gland, which often occurs together with pheochromocytomas. The serum calcium concentration is lowered only in a few cases. These patients usually suffer from diarrhea, so that intestinal malabsorption must be taken into account (TASHJIAN, 1968; CUNLIFFE, 1968). Medullary carcinoma of the thyroid can also be associated with mucosal neuromas, Marfanoid features, myopathy, and pigmentation, making the patients look like Giacometti sculptures (CUNLIFFE, 1970). Occasionally the disease is associated with Cushing's syndrome caused by ectopic production of ACTH (KEYNES, 1971). Elevated histaminase concentrations have been found in serum and in the tumor tissue, which accounts for the absence of any histamine reaction in some patients suffering from medullary carcinoma of the thyroid (BAYLIN, 1970; BAUM, 1971). In most of these patients hyperplasia of the parathyroids or an increased level of parathyroid hormone was recorded, which can be caused either by a minimal lowering of the serum calcium concentration or by a possible direct stimulation of parathyroid hormone secretion by CT (FISCHER, 1971). Medullary carcinomas are frequently familial with an autosomal dominant mode of inheritance. The life expectancy in patients subjected to surgery is good, and the single best laboratory examination in otherwise healthy subjects is radioimmunological determination of the CT concentration in the plasma (MELVIN, 1971). With the particular mode of inheritance half a family can be affected with the disease. Surgery is certainly the treatment of choice.

Overproduction of CT could be the cause of some cases of idiopathic "hypoparathyroidism" (FISCHER, 1967). An increased concentration of CT in the thyroid gland of these patients can be explained by the hypocalcemia and an inhibition of the release of CT into the plasma. Thyroidectomy does not cause any lasting correction of the hypocalcemia in patients with pseudohypoparathyroidism (LEE, 1968). Hyperostosis is demonstrable in one third of cases with idiopathic or pseudohypoparathyroidism. The hyperostosis is even more pronounced in hereditary osteopetrosis, which is occasionally associated with hypocalcemia and thus possibly with overproduction of CT or with hypersensitivity to CT (WHITE, 1965). The osteopetrosis was characteristic in the mice described by WALKER (1966) in which hypocalcemia, hypophosphatemia and a large number of parafollicular cells in the thyroid were indirect signs of overproduction of CT. There may be some connection between the CT and PTH secretions in patients with hyperparathyroidism with and without hypercalcemia and/or fibroosteoclasia. They can be explained by a dominance of parathyroid hormone and/or CT secretion (BINSWANGER, 1968).

In summary, the physiological importance of CT is still debatable. Thyroidectomy does not change the serum calcium concentration. In the absence of CT the serum calcium remains elevated for quite long periods following a calcium infusion; the thyroid protects rats from lethal effects due to parathyroid hormone (RASMUSSEN, 1967; WILLIAMS, 1966). Thyroidectomy does not alter the CT concentration in the human plasma (GUDMUNDSON, 1969). Animals entirely free of calcitonin have not been described. Nevertheless, CT is probably important for the rapid regulation of the serum calcium concentration. In patients with medullary carcinoma of the thyroid excess CT does not alter the histological or the radiological appearance of the skeleton.

c) Vitamin D

Vitamin D differs from parathyroid hormone and calcitonin (half-life 10–30 min) in that it has a half-life varying from days to months. According to conventional definitions, parathyroid hormone and calcitonin are *hormones* with a short half-life; they are excreted in inverse or direct proportion to the calcium concentration in the plasma. Vitamin D, however, must be supplied with the diet, and it is stored in the body. Parathyroid hormone and calcitonin are polypeptide hormones with a similar number of amino-acid residues and with molecular weights of the same order of magnitude. Their structure is species-specific, whereas this is not the case with vitamin D, a steroid vitamin or hormone effective in all the known vertebrates.

Vitamin D has several features in common with hormones, e.g. the stimulation of the synthesis of the biologically active 1,25-dihydroxycholecalciferol from vitamin D by low serum phosphate concentrations and para-

thyroid hormone (TANAKA, 1973; RASMUSSEN, 1972).

Vitamin D has two important sites of action, the small intestine and the skeleton. Hypocalcemia is normalized by the administration of vitamin D regardless of the pathogenesis, via increased absorption of calcium in the gut and probably via increased bone resorption. Vitamin-D deficiency leads to rickets in the child and in the adult to osteomalacia, which is often associated with oesteoporosis (WERNLY, 1952; GARNER, 1966). The main causes of osteomalacia in the adult are renal insufficiency and steatorrhea with malabsorption, which are associated with a reduction of protein absorption and of collagen synthesis. Severe osteomalacia rarely develops after gastrectomy. In renal insufficiency and in phosphate diabetes a resistance to the action of vitamin D is considered a cause of the osteomalacia. An isolated deficiency of vitamin D has only been demonstrated in the adult in severe nutritional deficiency, and has been connected with the lack of sunlight in England. No isolated disorder of vitamin-D absorption in the gut has been demonstrated so far (p. 865).

α) Uptake and Metabolism

The vitamins of the D group are steroids used in clinical practice in the form of ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3), or as dihydrotachysterol (calcamin, AT-10). The physiological properties of vitamin D_2 and D_3 and dihydrotachysterol are not very different.

Dihydrotachysterol has a shorter half-life than ergocalciferol (HARRISON, 1967). The different preparations are not discussed individually (p. 903). Unless otherwise stated, the properties of vitamin D_3 are described.

Vitamin D_3 is absorbed in the small intestine and subsequently transformed in the liver into 25-hydroxycholecalciferol (25-HCC) (PONCHON, 1969). 25-HCC is subsequently converted to the biologically active 1,25-dihydroxycholecalciferol (1,25-DHCC) in the kidney (FRASER and KODICELE, 1970). DELUCA and his collaborators (1972) have isolated and synthesized 25-HCC and 1,25-DHCC. Their clinical activity is being tested. Following the administration of 25-HCC and 1,25-DHCC the onset of action in the small intestine is earlier than that of vitamin D_3, since vitamin D_3 has to be transformed to 1,25-DHCC to become biologically active. The action of 1,25-DHCC on calcium absorption from the intestine in the rat starts several hours earlier than the action of vitamin D_3 (DELUCA, 1972). Infusion of 25-HCC into the mesenteric artery increases calcium absorption within the same time period, whereas vitamin D_3 in much higher doses remains ineffective (OLSON, 1969). In bone cell cultures 25-HCC and 1,25 DHCC increase calcium release, whereas vitamin D_3 produces only doubtful effects (RAISZ, 1972). Most of the experiments reported have been performed with vitamin D_3 as 1,25-DHCC is not yet easily available.

Vitamin D is absorbed in the small intestine. The minimum amount required to normalize the serum calcium in a growing rat fed on a diet with no vitamin D is less than 1 USP unit. The minimum requirement for man is not known. It is probably about 10 units per day. Vitamin D is also administered parenterally, particularly in intestinal malabsorption. It is poorly absorbed in the absence of bile in cases of bile obstruction, such as primary biliary cirrhosis and in steatorrhea, since it is a fat-soluble vitamin. The concentration of vitamin D in the plasma can only be roughly estimated biologically. It is determined by the measurement of the absorption of radioactive calcium by the gut, the increase in the serum calcium concentration, or the ash content and mineralization of the skeleton in rats or hens with rickets. Competitive protein-binding assays have been introduced for the measurement of vitamin D and 25-HCC in human serum (BELSEY, 1971; BAYARD, 1972).

Radioactive vitamin D_3 has a short half-life of 12 hours in the plasma but is converted into 25-hydroxycholecaliferol, whose half-life is 19.6 days (SMITH, 1971).

Vitamin D is stored for months in the liver and in the skin. Because of the long half-life there is a danger of vitamin-D accumulation and intoxication leading to hypercalcemia and nephrolithiasis, and of generalized deposition of calcium in blood vessels and soft tissue. 7-Dehydrocholesterol is converted to active vitamin D in the skin by the action of ultraviolet light. For this reason, patients with hypocalcemia and hypoparathyroidism need less vitamin D in summer than in winter. Exposure to sunlight can lead to hypercalcemia in patients with sarcoidosis since the compensatory fall of the calcium concentration in the plasma due to calcitonin is presumably not sufficient to normalize the serum calcium. Patients with sarcoidosis show increased sensitivity to vitamin D. The concentration in the plasma, measured biologically for its antirachitic activity, is within normal limits (THOMAS, 1959). Small amounts of vitamin D that are inactive in normal persons lead to hypercalcemia in patients with sarcoidosis (TAYLOR, 1963; BELL, 1964). In idiopathic hypercalcemia, the antirachitic activity is still elevated 14 months after discontinuation of

treatment with vitamin D; the breakdown is probably delayed (FELLER, 1958). The serum calcium and the intestinal calcium absorption are increased by 1,25-DHCC in patients in advanced renal failure (BRICKMAN, 1972), even if it is administered in a dose ineffective for vitamin D_3.

β) Sites of Action of Vitamin D

The most important sites of action are the small intestine and the bones, where vitamin D accumulates rapidly after a single injection (DELUCA, 1968). Physiological amounts (thought to be 10 units/day in man) primarily promote calcium absorption in the small intestine. Larger amounts (≃1 000–10 000 units/day) stimulate the mobilization of calcium from bones and lead to a rise in the serum calcium concentration. Toxic amounts of vitamin D or parathyroid hormone cause hypercalcemia, nephrocalcinosis, and azotemia. An exception is vitamin D-resistent rickets, which can be treated with over 500 000 units daily without necessarily giving rise to hypercalcemia.

Gastrointestinal Tract. Vitamin D in physiological concentrations primarily increases the uptake of calcium by the small intestine. The action of parathyroid hormone is less obvious than that of vitamin D. The calcium and phosphate absorption are directly stimulated by vitamin D. The maximum calcium absorption occurs in the duodenum, the maximum phosphate absorption in the jejunum (KOWARSKI, 1969). Calcium is necessary for vitamin D-dependent phosphate transport (HARRISON, 1961). calcium transport *in vivo* does not depend on the presence of phosphate (WALLING, 1969).

Increased calcium absorption under the influence of vitamin D can be demonstrated *in vitro* on isolated segments of the gut. The absorption is more pronounced when the segments are incubated with oxygen (SCHACHTER, 1961). However, a partial effect of vitamin D is still demonstrable at low temperatures and in the presence of cyanide or in a nitrogen atmosphere when oxidative metabolism is minimal (HARRISON, 1965). Part of the action of vitamin D is due to a rise in the permeability of the small intestine to calcium, but it is also actively absorbed under the influence of vitamin D depending on the presence of oxygen (SCHACHTER, 1966; WASSERMANN, 1968 a).

The primary sites of action of vitamin D in the intestinal mucosa may lie in the cell nucleus, since 1,25-DHCC is stored in the cell nuclei of the small intestine (TSAI, 1972). The synthesis of ribonucleic acids precedes the delayed onset of action of vitamin D on calcium absorption in the gut. The formation of ribonucleic acids is in turn followed after 16 hours by the release or synthesis of calcium-dependent adenosine triphosphatase and a calcium-binding protein (MELANCON, 1970; WASSERMANN, 1968 b). This action is probably regulated in the cell nucleus since the stimulation of the calcium absorption produced by the action of vitamin D and by parathyroid hormone is inhibited with actinomycin-D (CORRADINO, 1968) (p. 874).

The physiological importance of the calcium-binding protein or calcium-dependent adenosine triphosphatase for intestinal calcium absorption *in vivo* has not yet been demonstrated because selective inhibition is not possible.

Bones. Vitamin D probably leads to an increase in bone formation and in higher doses to increased bone resorption. In vitamin-D deficiency an increase of the osteoid mass and osteoporosis develop in association with pseudofractures or Looser's zones. In contrast to hypocalcemia caused by parathyroid hormone deficiency, vitamin-D deficiency or phosphate deficiency leads to a marked increase of osteoid, and it appears probable that vitamin D has a direct effect on the formation of new bone. This increase of osteoid results from accelerated collagen formation and reduced mineralization of the skeleton in the rachitic rat (ROHR, 1965; PATERSON, 1968). A similar increase in collagen synthesis is encountered in hyperparathyroidism. It may also be a sign of secondary hyperparathyroidism in patients suffering from osteomalacia (FLANAGAN, 1965).

In osteomalacia, there is a discrepancy between kinetic data measured with strontium or radioactive calcium and the histological findings (p. 864). The accretion and the calcium pool can be increased, whereas the rate of bone formation measured with tetracycline in bone biopsies is retarded (FRASER, 1960; FROST, 1963). The accretion or deposition of calcium and phosphate, measured isotopic-kinetically, is increased after treatment with vitamin D (BAUER, 1956; HARRIS, 1965). In rats, vitamin D leads to an increased incorporation of radioactive ^{45}Ca and radiolabeled phosphate into the bones (CARLSON, 1952) and stimulates growth, particularly the growth of the skeleton. The conversion of the epiphyseal cartilage into calcified apatite is taken as a biological measurement of the vitamin D activity of an unknown solution.

Administration of vitamin D to patients with chronic azotemia or intestinal malabsorption results in an increase in the number of osteo-

blasts, the storage of tetracycline, and the calcification of osteoid seams (BINSWANGER, 1968; BORDIER, 1968). In addition, vitamin D also causes an increased incorporation of phospholipids into the bones. This can be demonstrated histochemically at the transitional zones between osteoid and apatite (CRUESS, 1967; IRVING, 1959).

Vitamin D in higher doses leads to increased bone breakdown with a rise of the serum calcium concentration. This occurs even with a calcium-deficient diet. It is preceded by an elevated release of citrate, which can be interpreted as an indication that citrate in the plasma binds the calcium, thus leading to an increase in the calcium mobilization (CARLSON, 1954). The rise of citrate in the plasma is not a mandatory finding, since the liberation of calcium is also raised in rats treated with cortisone and vitamin D, without a change in the citrate concentration in the plasma (HARRISON, 1957). It is possible that the increased release of calcium inhibits aconitase and retards the citrate metabolism (p. 875).

The increased bone breakdown under the influence of vitamin D and parathyroid hormone is, like the increased calcium absorption in the small intestine, inhibited by actinomycin D and is probably under the control of the cell nucleus (HARRISON, 1966) (p. 11). Vitamin D *in vitro* causes an increase in the number of osteoclasts and in the demineralized bone matrix around osteocytes (GOLDHABER, 1965; BÉLANGER, 1965). The release of calcium from bone-cell cultures is accelerated by 1,25-DHCC whereas 25-HCC is effective in higher amounts only and vitamin D is ineffective (RAISZ, 1972).

Parathyroid hormone and vitamin D synergistically stimulate bone resorption. Parathyroid hormone leads to an increase in bone resorption only in the presence of vitamin D, whereas in rats, vitamin D leads to an increased excretion of hydroxyproline in the urine only when combined with parathyroid hormone (RASMUSSEN, 1963; PECHET, 1967).

Vitamin D stimulates bone breakdown and leads to the release of calcium into the plasma. At the same time, there is an improvement in the skeletal mineralization. The question as to whether the bone mineralization is increased secondary to an elevated calcium supply, or whereas in rats, vitamin D leads to an increased of new bone directly is still open. The latter view is supported by the fact that hypocalcemia alone, such as is found in hypoparathyroidism, is not asscociated with any considerable increase of the osteoid mass, in contrast to the findings in vitamin-D deficiency.

Kidneys. It is not known whether vitamin D in physiologic concentrations has a direct action on the renal tubules. During treatment of rickets or osteomalcia with vitamin D, there is a reduction of the phosphate excretion (HARRISON, 1941), associated with inhibition of parathyroid hormone secretion by the rise in the serum calcium concentration. In the absence of parathyroid hormone vitamin D leads to a slight rise in the phosphate excretion through the kidneys, but the phosphate clearance remains within normal limits (ALBRIGHT, 1948). The rise is in the same order as that obtained by EISENBERG (1965) during the treatment of hypoparathyroidism with calcium infusions and may be a result of the increased serum calcium concentration. In thyroparathyroidectomized dogs, vitamin D, 25-HCC and 1,25-DHCC cause a fall in fractional phosphate, sodium and calcium excretion (PASCHETT, 1972).

Vitamin-D intoxication leads to hypercalciuria in man, and in severe cases to hypercalcemia, nephrocalcinosis, nephrolithiasis and soft-tissue calcifications. A direct stimulatory effect on urinary calcium excretion is debatable. It might be the result of inhibition of parathyroid hormone secretion by a rise in the calcium absorption in the gut, of bone resorption and of a rise in the serum calcium concentration.

An increase in the magnesium excretion is observed in rats, together with a fall in the magnesium concentration in the plasma. This effect is in contrast to the action of parathyroid hormone, which inhibits the magnesium excretion (LIFSHITZ, 1967).

Apart from nephrocalcinosis, which arises in vitamin-D intoxication, no other actions of vitamin D on the kidneys can be demonstrated with certainty.

Mitochondria. Vitamin D promotes calcium uptake. This effect is inhibited by cortisone *in vitro* as it is *in vivo* (KIMBERG, 1969). Like parathyroid hormone, vitamin D stimulates the release of calcium from liver and kidney mitochondria (ENGSTROM, 1964) (p. 877).

6. Bones, Calcium and Phosphate

(Interrelationships and Conclusions)

There is a constant exchange of calcium between the bones and the extracellular fluid. Part of the calcium is exchanged according to physicochemical laws, and the remainder is incorporated into or released from the bones under the regulation of specific cells. Some of the bone mineral is not exchanged with the extracellular fluid for years. In adulthood, the formation of new bone is equal to its breakdown.

The serum calcium concentration is maintained within narrow limits under the influences of parathyroid hormone, calcitonin, and vitamin D. Bone formation and bone resorption are influenced by the homeostatic requirements of the plasma for calcium. An elevated serum calcium concentration is produced primarily by an increased release of calcium from the bones. When the calcium concentration falls, bone resorption is inhibited and/or increased amounts of calcium are incorporated into the bones. Small and rapid changes in the serum calcium concentration, which are important for the minute-by-minute regulation of the serum calcium concentration, are effected by alterations of the calcium absorption in the small intestine and by the reabsorption of calcium in the renal tubules (NORDIN, 1970).

a) Bones

Regulation of the serum calcium concentration is independent of the bone mass. Since the calcium reserves in the bones are unlimited, a calcium deficiency (osteoporosis) or increased calcium retention (hyperostosis) only becomes clinically and radiologically manifest after some months or even years. The most important sites for the regulation of the bone mass are the collagen synthesis, the mineralization of the bone matrix and bone resorption.

Vitamin D is necessary for bone growth, for skeletal mineralization, and to some extent for the release of calcium from the bones under the influence of parathyroid hormone. High concentrations of vitamin D stimulate bone breakdown. Amino acids must be available primarily for the formation of bone matrix. Calcium and phosphate are necessary for the mineralization of the skeleton. A sodium-deficient diet or diminished intestinal calcium absorption leads to osteoporosis in the growing rat and in patients with intestinal malabsorption. Phosphate and vitamin-D deficiency lead to a delayed mineralization of the skeleton with increased osteoid.

Skeletal growth and skeletal remodeling are affected by parathyroid hormone, calcitonin, vitamin D, and also by thyroxin, the adrenocortical steroids, the sex hormones and growth hormone. Thyroxin promotes bone remodeling, whereas the adrenocortical hormones inhibit it. Both produce osteoporosis, since the bone resorption exceeds the formation of bone. With estrogens, bone formation is usually inhibited to a lesser extent than bone resorption. Estrogens can thus prevent the osteoporosis from progressing at the most. Growth hormone increases the longitudinal growth of the bones by activating osteoblastic activity and bone resorption. An increase in the calcium concentration in the plasma indicates primarily an elevated bone resorption (Table 3). Hydroxyproline excretion in the urine parallels the formation of new bone and bone resorption. In most cases, a rise in hydroxyproline excretion in the urine is a sign of increased bone breakdown. Reduced and normal values overlap in groups of adult subjects; the excretion can only be compared in the same individual. Whenever possible, the calcium concentration in the plasma is maintained within normal limits and calcium is released from the bones, even when the skeleton can no longer tolerate the physical strain and bone pains or fractures develop.

b) Solubility of Calcium and Phosphate in the Plasma and Bones

There is a constant exchange of calcium between the bone surfaces and the extracellular fluid, and the sum of the calcium molecules in the two compartments remains unchanged. When the serum calcium falls, bone resorption, calcium absorption in the gut, and the reabsorption of calcium by the kidneys are increased and adjusted to the new conditions. Bone resorption and intestinal calcium absorption are inhibited in hypercalcemia, whereas the calcium excretion in the urine is increased. Compensatory processes independent of hormonal regulation come into action rapidly but are rapidly exhausted. The same is true to a lesser degree of the hormone-regulated processes in the renal tubules and the gut. Hypocalcemia or hypercalcemia of long duration is compensated by a cell-mediated, hormone-regulated incorporation or release of calcium from the bone. In the absence of parathyroid hormone and calcitonin the calcium concentration in the plasma is 4–7 mg/100 ml. When the parathyroids alone are missing in rats, the fall in the calcium concentration is greater than when the thyroid and parathyroids have been removed. This indicates that a minimal release of calcitonin continues in spite of the hypocalcemia. When the serum calcium falls below 6 mg/100 ml the exchange between the bone and plasma is chiefly a physicochemical process. Normalization of the serum calcium to 10 mg/100 ml involves metabolic regulation such as is produced by parathyroid hormone and/or vitamin D (MCLEAN, 1961).

In vitamin-D deficiency, the calcium phosphate product in the serum falls. Vitamin D is necessary for the increased release of calcium from the bones under the influence of parathyroid hormone. Parathyroid hormone has

no effect on the calcium × phosphate product as long as the urinary phosphate excretion remains normal in the absence of renal insufficiency. A rise in the serum calcium concentration leads to inhibition of parathyroid hormone secretion. A rise in the phosphate concentration in the plasma leads to a simultaneous fall in the calcium concentration and, together with an increased release of parathyroid hormone, to an increased renal phosphate excretion. This fact is important in potentially fatal hypercalcemia since an infusion of phosphate causes a prompt fall in the calcium concentration (HERBERT, 1966; KISTLER, 1966). This fall has also been demonstrated in parathyroidectomized patients. If parathyroid hormone is constantly administered to parathyroidectomized rats, the hydroxyproline excretion in the urine and the histologically detectable osteolysis around osteocytes are not altered by phosphate administration, so that improved mineralization is a possible effect (FEINBLATT, 1970). The calcium is not excreted in the urine or gut, but is retained or at least temporarily sequestered in the bones and in the intracellular fluid (EISENBERG, 1970) (Fig. 19). Phosphate infusions can lead to the precipitation of calcium phosphate at the infusion site and in soft tissue such as in the liver and the myocardium, especially in patients with renal insufficiency. Phosphate deficiency leads to osteomalacia and to hypercalciuria. Phosphate administration leads to increased retention of calcium in the bones (LOTZ, 1968; LUDWIG, 1967; GOLDSMITH, 1967; NAGENT DE DEUXCHAINES, 1967).

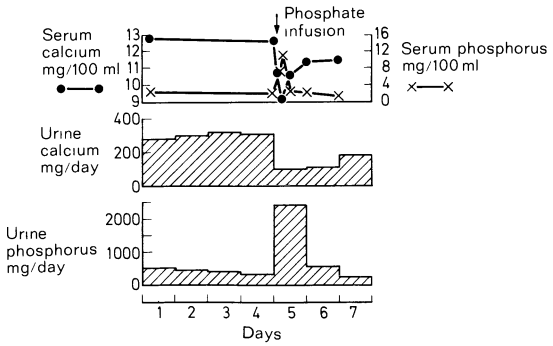


Fig. 19. Serum calcium and phosphate concentrations, and urinary calcium and phosphate excretions following an infusion of 3.065 g phosphorus over 6 h in a patient with primary hyperparathyroidism. (After KISTLER, 1966)

Phosphate and calcitonin may lead to inhibition of alkaline phosphatase or pyrophosphatase activity in bone, and thus lead to inhibition of the release of bone minerals and of bone resorption via a diminished dissolution of pyrophosphatases (Fig. 20) (FERNLEY, 1968; FLEISCH, 1970; RUSSELL, 1970) (p. 889). Calcium release from bone-cell cultures and bone resorption are inhibited by an increased phosphate concentration in the incubation medium (RAISZ, 1969). Bone resorption is also inhibited *in vivo* in the presence of phosphate even in parathyroidectomized animals, ruling out a stimulation of parthyroid hormone secretion in hypocalcemia (BINSWANGER, 1971; BAYLINK, 1971). Conversely, an extreme phosphate deficiency can make a fall in the serum calcium after parathyroidectomy impossible (COBURN, 1970). Phosphate is also used therapeutically for reducing the calcium excretion in the urine and for preventing renal stone formation.

Calcium administration leads to a fall in the serum phosphate concentration in thyroid- and parathyroidectomized patients, and increased amounts of calcium and phosphate are excreted through the kidneys (EISENBERG, 1968). A calcium infusion into the renal artery of the dog leads to a decrease in urinary phosphate excretion. A massive administration of calcium, particularly when vitamin D is given at the same time, leads to a rise in the calcium × phosphate product in the plasma and to precipitation of calcium phosphate in the reticuloendothelial system of the liver and spleen and in the kidneys, with a consequent decrease in glomerular filtration (LAVENDER, 1963; BINSWANGER, 1966). The calcification of soft tissue is also possible after administration of phosphate and calcium, particularly in the presence of impaired renal function. The precipitation is favored in alkaline regions, such as in the renal tubules and occasionally in the gastric mucosa.

In *in-vitro* conditions ressembling the situation in the plasma, calcium phosphate is precipitated when the solubility product of calcium × phosphate is increased. With a fall in the serum calcium concentration following phosphate infusions, calcium is not excreted in the urine or in the stools. The maximum solubility product *in vitro* corresponds to the situation in vivo (HERBERT, 1966). An excessive solubility product of calcium × phosphate leads to deposition of calcium and phosphate in the bone and in soft tissue, while a fall in the calcium × phosphate product leads to a release of calcium and phosphate from the bones. The mechanisms underlying the fall of the calcium concentration following the administration of phosphate and those responsible for the fall of the phosphate concentration following calcium administration are presumably partly physicochemical, since they are not influenced by thyroparathyroidectomy. Physicochemical processes regulate the serum calcium concentration in the plasma continuously, whereas

the metabolic and the endocrine regulatory mechanisms come into action within minutes or days, depending on the degree of compensation required. The later mechanisms are less rapidly exhausted.

Parathyroid hormone increases the calcium concentration and leads to a fall in the phosphate concentration in the plasma via an increase in urinary phosphate excretion. An impairment of renal function usually causes a rise in phosphate and a fall in calcium in the plasma. This leads to an increase in osteoid mass for known (acidosis, vitamin-D resistance) or unknown reasons, even in the presence of a normal calcium × phosphate product.

The administration of calcitonin causes the calcium × phosphate product to fall by inhibiting bone resorption and stimulating the urinary phosphate excretion. A considerable fall can be seen in vitamin-D deficiency in intestinal malabsorption, where the absorption of calcium and phosphate is reduced. The hypocalcemia leads to secondary hyperparathyroidism and thus to an additional fall of the phosphate concentration in the serum. Growth hormone increases the calcium × phosphate product. It is raised in children and in acromegaly.

c) Regulation of the Serum Calcium Concentration

Vitamin D and calcitonin have been demonstrated in all vertebrates including fish, whereas parathyroid hormone appears for the first time phylogenetically in the amphibians (Copp, 1970). Seawater contains 10 mMol/liter calcium, as against a concentration of 2.5 mMol calcium in the plasma. It is reasonable to assume that parathyroid hormone is not necessary for the mobilization of calcium from the skeleton in the sea, whereas in the amphibians, which leave the water environment, calcium is stored in the bones and must be released under the influence of parathyroid hormone for the maintenance of a normal calcium concentration in the plasma. Calcitonin is necessary in fish for protection from the high-calcium environment occurring in the sea. In higher vertebrates, parathyroid hormone is important in the maintenance of the calcium concentration in the plasma, whereas vitamin D is essential for the mineralization of the skeleton and calcitonin for the fall and rapid changes in the serum calcium concentration. Although only a small proportion of the calcium is found in the plasma, this is maintained within the same narrow limits in the presence of a changing intake and excretion. A rise in the serum calcium concentration is observed with an increase in bone resorption caused by thyroxin and parathyroid hormone. A fall is encountered when bone resorption is inhibited by calcitonin. Hypocalcemia can also be a sign of an increase in the skeletal mineralization at the beginning of the treatment of rickets with vitamin D or as an initial effect of parathyroid hormone (Table 3). The significance of glucagon as a calcium-lowering hormone is still questioned because pharmalogical amounts have to be used: glucagon leads through an increased release of calcitonin to a fall in the calcium and phosphate concentrations in the plasma (Avioli, 1969). Independently, glucagon inhibits bone resorption *in vitro* and leads to hypocalcemia in thyroidectomized rats (Skin, 1970). Like calcitonin, it appears to have a direct effect on bone. Vitamin D leads to normalization of the calcium concentration in the plasma in hypocalcemias of any etiology. Precise physiologic regulation of the serum calcium concentration is achieved by the counteraction of the calcium-increasing para-

Table 3. The bones and the regulation of the serum calcium concentration

	Bone density (X-ray)	Morphometry		Serum calcium	Urinary hydroxyproline
		Bone formation	Bone resorption		
Parathyroid hormone	→ to ↓	↓ in vitro ↑ in vivo	↑	↑	↑
Calcitonin	→ to ↑	?	↓	↓	↓
Growth hormone	↑	↑	↑	→ to (↑)	↑
Thyroxine	↓	(↑)	↑	→ to ↑	↑
Cortisone	↓	↓	(↓)	→ to ↓	↓
Estrogens	→	(↓)	↓	→	↓
Vitamin D	↑	↑	↑	→ to ↑	?
Senile osteoporosis	↓	(↓) or (↑)	↑	→	→
Calcium	→	→	↓(PTH↓)	→ to ↑	↓
Phosphate	→ to ↑	↑	↓	↓	→

thyroid hormone and the calcium-lowering calcitonin. The action of parathyroid hormone is *qualitatively* similar to that of vitamin D. Parathyroid hormone has a rapid onset of action (within minutes) in comparison to vitamin D and its biologically active metabolites (within hours). Intestinal calcium absorption is promoted primarily by vitamin D, whereas the release of calcium from bones is increased prodominantly by parathyroid hormone. A rise in the serum calcium (due to calcium, parathyroid hormone, or vitamin D) inhibits the phosphate reabsorption in the renal tubules (EISENBERG, 1968). Calcitonin stimulates the renal excretion of phosphate despite the hypocalcemia. Phosphate excretion is inhibited in renal insufficiency or when calcium is infused directly into the renal artery of dogs (LAVENDER, 1963).

Parathyroid hormone is the single most important agent in the regulation of the serum calcium concentration. Parathyroid hormone deficiency after parathyroidectomy leads to hypocalcemia, whereas thyroidectomy and calcitonin deficiency cause no rise in the calcium concentration in man.

Regulation of the calcium concentration in the plasma is accomplished by means of variations in the calcium absorption by the small intestine and in the calcium reabsorption in the renal tubules, and also by means of changes in the rates of incorporation and release of calcium around the osteocytes and the formation of osteoblasts and osteoclasts. Parathyroid hormone causes a rise in the serum calcium concentration via an increase in the calcium absorption through the gut, the reabsorption in the renal tubules, and raised phosphate excretion. The incorporation and release of calcium in the bones may be regulated by osteocytes; a halo is characteristically formed around the osteocytes, reflecting increased bone breakdown and thus a release of calcium. The osteocytic bone resorption is increased under the influence of parathyroid hormone and inhibited by calcitonin (RASMUSSEN, 1967). The osteocytes may thus be partly responsible for the regulation of the calcium level in the plasma. Presumably, alkaline phosphatase in the osteocytes corresponds to the pyrophosphatase which promotes the dissolution of pyrophosphates. Pyrophosphates protect apatite from dissolution (FLEISCH, 1966). Parathyroid hormone causes a histochemically demonstrable increase of the alkaline phosphatase in the osteocytes, which may correspond to the pyrophosphatase. It has been postulated that there is an increase in pyrophosphatase activity and in the breakdown of pyrophosphate under the influence of parathyroid hormone, which favors the dissolution of bone mineral (RASMUSSEN, 1967; FLEISCH, 1970; RUSSELL, 1970) (Fig. 20).

If this hypothesis is correct, the administration of pyrophosphates should lead to a fall in the calcium concentration in the serum, but this has not been observed, since pyrophosphates are immediately metabolized by a pyrophosphatase. Because of this, analogous compounds have been synthesized, such as the phosphonates, which are not broken down by pyrophosphatase. Phosphonates inhibit osteolysis stimulated by parathyroid hormone *in vitro* in mice calvariae and they also lead to a fall in the calcium concentration in parathyroidectomized rats treated with parathyroid hormone (FLEISCH, 1968b, 1969).

The calcium-lowering action of calcitonin occurs within minutes. It can only be seen

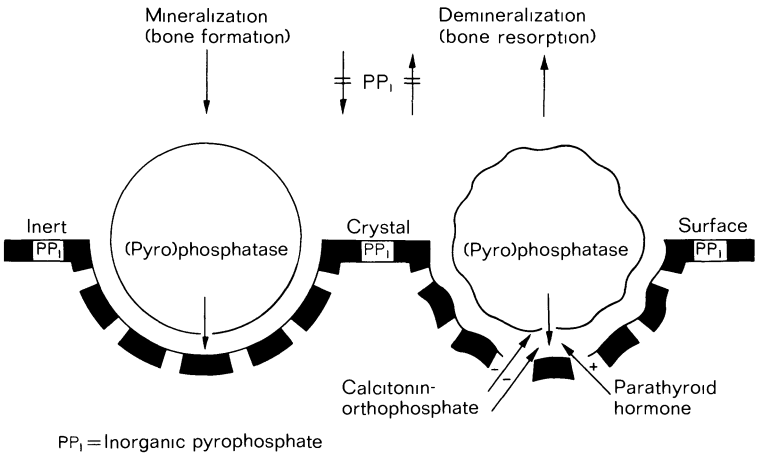


Fig. 20. Hypothetical role of pyrophosphate and pyrophosphatase, and parathyroid hormone, calcitonin, and phosphate in bone mineralization and resorption. It is proposed that mineralization and resorption occur more readily after hydrolysis of the pyrophosphate layer by pyrophosphatase. (After RUSSELL, 1970)

when the bone resorption is increased. It is possible that calcitonin is necessary for the rapid regulation of the serum calcium concentration, as a rise of the serum calcium above the normal level under the influence of parathyroid hormone is seen only after one hour. Calcitonin and parathyroid hormone act independently of each other. Calcitonin is effective in parathyroidectomized animals, and parathyroid hormone in thyroidectomized animals (GUDMUNDSON, 1966). Calcitonin inhibits an increase in the serum calcium concentration regardless of the cause (e.g. parathyroid hormone, 3′,5′-AMP, vitamin D). It does not have to be an antagonist acting at the same biological site. Calcitonin causes a rapid fall in the calcium concentration in the serum ($t_{\frac{1}{2}} < 30$ min); a slow fall is seen following parathyroidectomy ($t_{\frac{1}{2}} > 2$ hours), although the concentration of parathyroid hormone in the serum falls by 50% even after as little as 10–20 min (TASHJIAN, 1966; MELICK, 1965).

Actinomycin-D, an inhibitor of the messenger ribonucleic acids and thus of protein synthesis, impairs the release of calcium from the bones which occurs under the influence of parathyroid hormone and vitamin D. Vitamin D is essential for the full effect of parathyroid hormone. In contrast, vitamin D and actinomycin-D have no effect on the calcium-lowering action of calcitonin or on the rise in urinary phosphate excretion resulting from the action of parathyroid hormone (TASHJIAN, 1965; ARNAUD, 1966; DELUCA, 1968). These effects occur in the absence of previous protein synthesis, and are thus of rapid onset (within minutes), but they are also rapidly exhausted. The increase in osteoclasts due to parathyroid hormone requires time (some hours) and the previous synthesis of proteins. When protein synthesis and calcium release are in progress, the unlimited calcium reserves of the skeleton are utilized. Maintenance of a serum calcium concentration at a level of 10 mg/100 ml requires the constant secretion of parathyroid hormone and an adequate nutritional intake of vitamin D.

D. Hypoparathyroidism

B. COURVOISIER

1. Etiology and Pathogenesis

a) Postoperative Hypoparathyroidism

The most common cause of hypoparathyroidism is damage to one or more of the parathyroid glands during operations on the thyroid gland. Even with a flawless technique, this occurs in around 1–4% of cases. Damage results either from inadvertent removal of one or more of the glands or from ligation of an artery. From experiments in animals it has been deduced that more than half the parathyroid tissue must be lost before lasting insufficiency arises.

The parathyroids are endangered mainly during removal of large goitrous nodules because of their small size and also because they are often atypically situated. Topography of the arterial vascular supply and the possibilities and risks of arterial damage have been dealt with on p. 846. Postoperative hypoparathyroidism is observed considerably more frequently in females. It is assumed that there is some connection with the fact that thyroidectomies are performed more frequently in women than in men (hyperthyroidism is more common in females) or with the increased stress on the parathyroids associated with the variations of estrogen production during the menstrual cycle and pregnancy.

b) Hypoparathyroidism Following Treatment with Radioactive Iodine

Possibility of damage of this nature has been considered and examined both clinically and experimentally, but appears rather unlikely (EIPE, 1968).

c) Reactive Hypoparathyroidism after Removal of a Parathyroid Adenoma

The parathyroid adenoma leads via hypercalcemia to inactivity and atrophy of the remaining parathyroids. Removal of the adenoma can but need not necessarily lead to the development of temporary hypocalcemia. It may be that the atrophic parathyroids are incapable of producing sufficient hormone, or recalcification of the skeleton commences, or the compensatory excessive secretion of calcitonin leads to hypocalcemia. The fact that postoperative hypocalcemia is almost exclusively observed in primary hyperparathyroidism with skeletal involvement and practically never in the absence of skeletal involvement supports the idea of skeletal avidity for calcium and phosphate and thus suggests "recalcification tetany". The fact that the level of the serum phosphate is usually not elevated but lowered fits in with this interpretation.

d) Hypoparathyroidism in Newborns Associated with Hyperparathyroidism in the Mothers

Reactive, transitory parathyroid insufficiency is observed occasionally in the newborn due

to primary hyperparathyroidism in the mother. In a few cases, the condition in the mother is diagnosed for the first time when the disorder is found in the newborn.

e) Transitory, Congenital Hypoparathyroidism in Newborns

This form of parathyroid insufficiency arises between the 2nd and 19th days of life and is also observed in the absence of maternal hyperparathyroidism; according to FANCONI (1967, 1970) it may be due to a functional insufficiency of the parathyroids which are subjected to particularly severe stress during this phase of life (immaturity of parathyroid function, immaturity of glomerular function resulting in insufficiency of phosphate glomerular filtration, increased supply of phosphates from cow's milk). Hyperparathyroidism must always be carefully searched for in the mother and excluded, if necessary by observation over several years.

f) Idiopathic Hypoparathyroidism

This term applies to conditions which show all the biochemical and clinical symptoms of postoperative parathyroid insufficiency although no cause can be found. Pathogenesis and diagnosis of these disorders are discussed later on p. 899. In contrast to pseudohypoparathyroidism, where the immunoreactive parathyroid hormone concentration in the serum (IPTH) is usually elevated, IPTH is not measurable in idiopathic or surgical hypoparathyroidism.

2. Symptoms

a) Tetany

This is the best known and most important manifestation of hypocalcemia of parathyroid insufficiency. Clinically, it is a syndrome embracing neuromuscular overexcitability. Three subtypes are differentiated, each of which can appear in isolation, but all three can occur simultaneously in the same subject. These are: 1. the tetanic attack, 2. the tetanic equivalent, and 3. latent tetany.

b) The Tetanic Attack

This is associated with sensory-motor excitability and has a specific topographic and chronological course. The attack can arise spontaneously or can be precipitated by a mechanical, acoustic, or psychological stimulus or by hyperventilation. In the child, it is often observed following a febrile infection. The onset can be sudden and unexpected, but as a rule an aura precedes the event, the most common signs being fear, general malaise, muscular pains, paresthesias in the face and limbs and vasomotor disturbances. The duration of the aura varies widely from individual to individual. During the actual attack, there is tonic contraction of separate groups of muscles. The thumb and fingers are usually the first to be affected. The thumb is usually forced into the adduction position, the fingers are pressed together with the metacarpophalangeal joints flexed and the distal joints extended (obstetrician's hand). The hand is sometimes even maintained in complete flexion (tight fist) however; this is observed particularly in children. During the main crisis, the spasm extends from the hands to the arms, which lie adducted against the body with the elbows flexed. The legs are fixed in complete extension in the varus position with flexion of the foot and toes. The face often shows contraction of the upper lip accompanied by drooping of the corners of the mouth (carp mouth). A common symptom which PRADER describes in the child is the inability to show the teeth. Strabismus is occasionally also observed. The muscular cramps are usually extremely painful. They are accompanied by feelings of fear and paresthesias.

The autonomic nervous system can be involved during the crisis. This involvement can present, for example, as laryngospasm, which is particularly dangerous in the baby and young infant, and as spasm of the bronchi, cardia and sphincter of the bladder. In a similar manner, blood vessels are also sometimes affected by the crisis, causing RAYNAND's phenomenon, migraine, angina pectoris, etc. to occur. Autonomic and vascular tetanic features can completely dominate the clinical picture (visceral, vascular tetany) and in such cases the diagnosis is often rather difficult. Generalized convulsions and loss of consciousness are common in the child.

The crisis lasts for a few seconds, hours, or days, during which there are quite long remissions of the acute features. When the crisis stops, symptoms disappear in the reverse order to that in which they appeared: peripheral spasm is the last to recede.

c) Tetanic Equivalent, the Epileptic Seizure

The visceral and vascular forms of tetany mentioned above actually belong here when they arise in isolation. It is, however, more justifiable to speak of a tetanic equivalent with an epileptic seizure within the field of

tetany. It can be the only symptom of parathyroid insufficiency or it can arise with other tetanic manifestations. It cannot be differentiated from epilepsy clinically or by means of an electroencephalogram. Tetany occurring exclusively as part of the epileptic picture is most commonly observed in idiopathic hypoparathyroidism and idiopathic hypocalcemia, less frequently with postoperative hypoparathyroidism, but only rarely with hypocalcemia of any other etiology (D-hypovitaminosis, malabsorption). Signs of the aura and sphincter insufficiency can be present or absent. Occasionally, discrete features of latent tetany follow the attack. In the literature, cases are described which progressed as classic epilepsy for years and were treated as such until hypoparathyroidism was finally diagnosed either by chance or from the development of other tetanic symptoms. From this, it can be seen that routine estimation of calcium is essential in every case of epilepsy.

d) Latent Tetany

Latent tetany signifies a state in which a partial or complete acute attack can readily be provoked by diagnostic measures. This state of latent tetany embraces a series of neurovegetative and neuropsychiatric symptoms. They vary widely from one individual to the next and are not only seen with hypoparathyroidism but also with other forms of tetany. A long list of diagnostic procedures has been suggested for the recognition of latent tetany, and the most important of these are presented here.

α) Chvostek's Sign

Tapping of the facial nerve immediately below and in front of the ear (root of the facial nerve) results in twitching of the corner of the mouth, the nostril and the orbicularis oculi in latent tetany. Similar twitching can be elicited by percussion below the zygomatic arch at the midpoint between the corner of the mouth and the ear. Only simultaneous twitching of all three muscles after tapping the root of the facial nerve can be interpreted as a sign of tetany (Chvostek I). Twitching which can be released only in the region below the zygomatic arch or in the region of the cheek, or which appears only in two muscle groups after tapping the root of the facial nerve (defined as Chvostek II and III by different authors) can also arise in healthy subjects. Thus, such twitching is only of slight diagnostic value and must be used with caution. Occasionally, however, when the diagnosis of tetany has been confirmed, it can be useful in assessment of the course of the ilness, e.g. for estimating a therapeutic measure. Occasionally, Chvostek's facial phenomenon cannot be released in confirmed cases of hypoparathyroidism with greatly reduced serum calcium levels.

β) Trousseau's Sign

A sphygmomanometer is applied to the upper arm and a pressure just above the systolic blood pressure is exerted for 3 min. In tetanic subjects, a typical carpal spasm usually arises. Occasionally, this sign fails to occur in confirmed cases of tetany. In other cases the spasm can be induced by a presure much lower than the systolic pressure or simply through pressure on the nerve root in the medial bicipital groove. Finally, the spasm may be observed only after removal of the sphygmomanometer.

γ) Hyperventilation Test

Any form of latent tetany can become manifest in the presence of hyperventilation. After a few minutes, typical carpal spasm arises. A hyperventilation test carried out over 5 min may occasionally be negative, however, in mild forms of hypoparathyroidism, and is more commonly positive in nonendocrine, normocalcemic hyperventilation tetany. Carpal spasm can be evoked particularly easily by using Trousseau's arm constriction in combination with the hyperventilation test. It is best to apply the sphygmomanometer after the hyperventilation.

δ) Electrical Investigations (no longer used for practical purposes)

The excitability of motor nerves is raised in tetany of every etiology. With modern electromyographic methods, which, however, are available only to specialists, a series of changes more or less specific to tetany can be differentiated. Characteristic changes of the so-called twitching form are described as Erb's sign.

e) Course of Tetany

There are numerous individual variations. A tetanic attack can be solitary or can recur at more or less regular intervals. Between attacks, the patient usually shows subjective and objective signs of neuromuscular overexcitability, which are termed as latent tetany. In a few cases, the tetany remains latent and never progresses into acute tetany.

f) Pathology, Physiology, and Pathogenesis of Tetany

In spite of numerous investigations, the pathogenesis of tetany still remains uncertain. There is even controversy about the true site of origin of the tetanic phenomenon. From close analysis of the symptoms, it can be deduced that nervous overexcitability is present, involving the cerebral centers to a large degree, as well as the spinal cord and peripheral nerves. The role of calcium and/or magnesium and other electrolytes important for neuromuscular excitability has been discussed in the chapter on Physiology on p. 848. It has now been definitely established, however, that hypocalcemia is observed not only in hypoparathyroidism but also in a series of other disorders. This question has been dealt with under differential diagnosis (p. 897). It must be emphasized that tetany is a syndrome that can have very different reasons. Thus, tetany is *not* equivalent to parathyroid insufficiency, but only one of its manifestations. The habit of naming an illness after its main symptom (tetany = parathyroid insufficiency etc.) should be avoided.

Since tetany also occurs in the absence of parathyroid insufficiency, it is also important to know that the syndrome of nervous overexcitability is most marked in parathyroid insufficiency. This becomes manifest as severe tetanic attacks, and also as the epileptic seizure (which is not seen with normocalcemic tetany and with hypocalcemia due to D-hypovitaminosis or malabsorption), and as diverse neurological and psychopathologic features. Finally, the duration of the latent tetany can be prolonged, extending over decades.

g) Mental Changes

Mental changes occur in chronic hypocalcemia much more commonly than was suspected in the past. They are now usually recognized from their unspecific character even were other tetanic symptoms are absent, however. Thus, in the presence of vague mental disorders, the diagnosis of hypoparathyroidism should be considered more frequently that it has been in the past, and features of latent tetany should be searched for and serum calcium and phosphorus estimated.

In the child, untreated hypoparathyroidism and other forms of hypocalcemia (rickets, malabsorption, uremia) often lead to tetanic spasm (spasmophilia) but tetanic symptoms may sometimes be completely absent. In such cases, more definite neurological and mental symptoms are found, generalized malaise, photophobia and blepharospasm being the features most commonly observed. These children very often also show retardation of mental development, disorders of emotional adjustment and irritability and poor concentration in school. All symptoms disappear rapidly after correction of the hypocalcemia. The great responsibility incumbent on the doctor to recognize these cases early is obvious. It can without hesitation be ranked in importance with the problem of early diagnosis of infantile hypothyroidism with mental developmental disturbances.

Acute tetany and symptoms of latent tetany occur less frequently in adults with hypoparathyroidism than in children. Such patients are noticed because of their lability, anxiety, irritability, and lack of compensation. They are generally somewhat depressed and very often complain of chronic headaches. These are features of the endocrine psychosyndrome, which is dealt with in more detail in the section on hyperparathyroidism (BLEULER). In severe tetany, features of the acute exogenous reaction type (BONHÖFFER) are rarely observed, and "neuropathic crises" have been reported in the literature. As is true of all psychopathologic disorders occurring with endocrine illnesses, these symptoms are not specific or particularly characteristic of hypoparathyroidism. In contrast to previous opinions that psychic disorders occurred predominantly with idiopathic hypoparathyroidism, it has been shown in the last few years that these disturbances arise more frequently than was suspected in a discrete form after thyroidectomy also, and may occasionally develop into acute psychotic crises.

h) Neurologic Changes

As a rule, neurologic findings are normal in parathyroid insufficiency. There are, however, known cases of idiopathic or postoperative hypoparathyroidism with cerebral edema, increased intracranial pressure, papilledema and symptoms of a unilateral focal lesion which can simulate a brain tumur. Headache without neurologic findings is very common. In addition, extrapyramidal symptoms with Parkinsonism have been described in idiopathic hypoparathyroidism. Such disturbances can occasionally be accentuated or be caused by phenothiazine preparations (e.g. prochlorperazine). Mild features of Parkinsonism can even arise after oral treatment, whereas intramuscular injection (e.g. 10 mg prochlorperazine) can produce very severe neurodysleptic (dystonic) attacks. The attack begins with painful stiffness of the neck, paresthesia, fascicular muscular twitching, sweating and tachycardia, followed shortly after-

wards by tonic muscular cramps in the neck, jaws, tongue and extremities. Visual spasm and laryngospasm also develop and in addition, a series of "typical tetanic" features, such as obstetrician's hand, carpal spasm, Trousseau's and Chvostek's phenomena, may be found. This picture shows very marked similarities to the tetanic attack, but differs in that it does not respond at all, or at least not promptly, to treatment with calcium and magnesium but is arrested at once on intravenous administration of barbiturates. Refer to the next section for possible connections with cerebral calcifications.

i) Cerebral Calcifications

X-rays of the skull often show symmetric and small calcified foci irregularly distributed in the ganglia of the brain stem, particularly in the putamen and the nucleus caudatus. These calcifications can be distinguished from those of the choroid plexus by the fact that they extend further frontally in the brain, whereas those in the choroid plexus are limited to the region around the genu of the lateral ventricle. Their localization can be more precisely traced by means of sterographic X-rays. When they are very pronounced, they form opaque stripes or spots. Small calcified foci are occasionally also found in the cerebellum or in the deeper regions of the cortex of the brain. About 40 such cases of cerebral dystrophy have been observed and they have been encountered predominantly in idiopathic hypoparathyroidism and less commonly in chronic parathyroid insufficiency after thyroidectomy. They are not specific to hypoparathyroidism and are also observed in various congenital disorders of the nervous system including mongoloid idiocy and a familial form of oligophrenia. Finally, they account for some of the characteristic brain lesions found in endemic cretinism. The histological structure consists of predominantly perivascular hyaline masses, with secondary deposition of calcium and iron salts (pseudocalcium concrements). These changes are thus quite different from calcium metastases localized elsewhere and associated with hyperparathyroidism and hypercalcemia of any other etiology. It is not certain whether these dystrophic calcifications affect brain function. Nevertheless, it is suspected that the extremely severe reaction of certain patients with hypoparathyroidism to phenothiazine drugs (Parkinsonism, neurodysleptic attack, p. 893), could be due to irreversible damage to the extrapyramidal centers which cannot be influenced by treatment with calcium.

j) Cataract and Other Changes in the Eye

The cataract is the best known dystrophic manifestation of hypoparathyroidism. It is a direct sequela of chronic hypocalcemia and is observed in the idiopathic as well as the postoperative form of hypoparathyroidism. It is also seen, but less commonly, in chronic hypocalcemia of other etiologies. It is seen both in adults and in children. Often it is the key to diagnosing hypoparathyroidism in the age groups where cataracts are not normally found. In the early stages, it can be differentiated with no difficulty from other forms of cataracts, with the exception of those found in dystrophic myotonia; it is always bilateral, and is situated in the anterior and posterior cortical layers of the lens in the adult. Zonular and central cataracts are occasionally also observed in children. In advanced stages, clouding of the lens becomes diffuse and the nucleus of the lens also becomes involved, so that differentiation from other cataracts, e.g. senile cataract, is no longer possible. The rate of development varies greatly. Cases of bilateral tetanic cataracts have been described where blindness has resulted within months. In other cases, mild changes remain almost constant for years. A cataract, once formed, can cease to grow with suitable treatment, but it can never be cured. Sometimes further progress is observed even despite correct treatment. Every case of hypocalcemia should be examined for cataracts by a specialist early, to avoid recognition of the tetanic cataract only after loss of vision. At this stage, it is too late for effective therapy.

Up to now, there is no satisfactory explanation for the pathogenesis of the cataracts.

Papilledema, keratitis, strabismus, diplopia, lid ptosis, conjunctivitis, blepharitis etc. (Pohjola, 1962) are uncommon symptoms in idiopathic hypoparathyroidism.

k) Trophic Disorders of the Skin, Nails and Hair

These disorders are found predominantly in idiopathic hypocalcemia but have also been observed in long-standing untreated cases of postoperative hypoparathyroidism.

Hair. In hypoparathyroidism, tufts of hair can be lost, or complete alopecia may develop or the growth of hair may be sparse. In addition to the hair on the head, the entire body hair and the eyebrows and eyelashes are sometimes lost. Acute tetanic attacks are occasionally preceded by acute loss of hair.

Skin. In hypoparathyroidism, the skin is dry, split and scaly. Eczema can heal within a short time after correction of the hypocalcemia.

Nails. The nails show transverse furrows and are brittle. The skin often covers the nail fold and cornification is disturbed. This sometimes causes the nail to be rejected, and subsequent growth is incomplete.

Moniliasis. Infection with monilia albicans is very common in hypoparathyroidism. The nails and the corners of the mouth are the most frequent sites, but in severe cases the infection spreads over the whole of the skin and the buccal cavity and intestinal tract may also be affected. Changes in the hair, skin and nails can usually be corrected with adequate treatment of the hypoparathyroidism, but monilia infection of the nails does not respond to this sort of treatment.

l) Dental Damage

If hypoparathyroidism arises during growth, changes in the dental enamel are possible. The teeth then show transverse grooves with hypoplasia of or faults in the enamel, each groove corresponding to a tetanic episode during the phase of development of this part of the tooth. In addition to the faults in the enamel, the whole process of development and growth of the teeth can be disturbed. The pulpa is abnormally extensive, the root is shortened and conical, dental eruption is delayed or fails to occur, and dental stumps showing no true formal differentiation are occasionally found enclosed within the jaw bones. The cortex of the alveolae is sometimes thickened (lamina dura) (in constrast to the findings in hyperparathyroidism where it has been known to disappear).

Like cataracts, the faults in the enamel are due to the hypocalcemia, as is borne out by the fact that similar defects are seen in children with rickets due to hypocalcemia, but it is still not known for certain whether the other irregularities of dental formation and growth are a result of the parathyroid insufficiency or are malformations.

m) Bone and Joint Changes

Hyperostosis of the skeleton is observed in one third of cases of idiopathic hypoparathyroidism and very occasionally in postoperative hypoparathyroidism (MARTIN, 1966) in contrast to the bone atrophy in hyperparathyroidism. Localization and extension vary widely: hyperostosis can occur exclusively on the long bones, jaw bones and skull (generalized thickening of the cranium) or it can involve the entire skeleton, including extremities, vertebral column and pelvis. The change usually takes the form of periostosis and seldom that of full hyperostosis (periostosis and endostosis). Radiologically, the skeleton appears abnormally dense and the periosteum is thickened due to bone apposition. These skeletal changes are never very marked and they do not tend to progress rapidly. There is never a state of overmineralization such as is found in osteopetrosis. Histological examination reveals thickening of the corticalis and spongiosal trabeculae without signs of increased activity. More frequently, there are signs of reduced osteoclastic activity.

In some cases, bone changes are characterized by the combination of periostal hypertrophy and endostal "pseudoatrophy", where the spongiosal trabeculae, although larger in diameter, are reduced in number.

Painful joint changes are often found in hypoparathyroidism whether osteopathy is present or not. They arise in the vertebral column and in the ileosacral and hip joints. Inflammatory features do not arise, but osteophytic formation with calcification is often severe in the capsules, tendons and surrounding soft tissues. Changes in the ileosacral joints can be distinguished from those in Bechterew's disease by the extensive para-articular hyperostosis.

n) Biochemical Changes and Laboratory Findings

Radioimmunological methods have made it possible to demonstrate elevated parathyroid hormone levels in the blood of patients with hyperparathyroidism (p. 871). Unfortunately, this test cannot yet be used for the detection of diminished hormone levels in the blood. However, pseudohypoparathyroidism with an increased concentration of immunoreactive parathyroid hormone (IPTH) can be clearly differentiated from idiopathic or postoperative hypoparathyriodism; in these conditions the IPTH level is too low for assay. The diagnosis of hypoparathyroidism must thus be made indirectly for the time being, on the basis of biochemical effects caused by the hormone deficiency.

The classic humoral syndrome of hypoparathyroidism is characterized by the triad hypocalcemia, hyperphosphatemia and hypocalciuria. Hypocalcemia is the most constant and important change which is almost exclusively responsible for the clinical and morphological symptoms. The serum calcium rarely falls below 6 mg/100 ml. Serum phosphate is elevated to above 5 mg/100 ml in adults and above

7 mg/100 ml in children. Finally, the 24-hour urinary excretion of calcium is reduced to 10–50 mg. The 24-hour urinary excretion of phosphate, on the other hand, can vary so widely (p. 854) that it is of no use for the diagnosis. The serum alkaline phosphatase is always normal. This is important in the differentiation from malabsorption and rachitic hypocalcemia, where the phosphatase is increased in the majority of cases. Hyperuricemia is found in a few cases of idiopathic hypoparathyroidism.

ECG. A Q-T segment with a prolonged isoelectric S-T and very short T wave is characteristic of every form of hypocalcemia.

EEG. The EEG is frequently changed in chronic hypoparathyroidism. The most common deviation consists of long waves (2–5 per sec) arising in groups or singly, accompanied by detached scatterings of spikes. During tetanic attacks, spastic potentials, often of cirumscribed localization, are sometimes recorded. All changes are accentuated by hyperventilation, and terminated by correction of the hypocalcemia. We have seen that the epileptic seizure is a symptom of tetany but it is impossible to differentiate it from more common forms of epilepsy either by the clinical findings or by means of the EEG. Cerebrospinal fluid is normal, but the pressure is occasionally raised.

o) Onset and Course of the Disorder

The clinical features of hypoparathyroidism are multiple. Postoperative hypoparathyroidism usually sets in as early as 24–48 hours after surgery (thyroidectomy or removal of a parathyroid adenoma) and usually starts with a typical attack of tetany. The hypocalcemia is usually marked at this point. If the case is not treated, latent tetany then develops, with occasional mild or severe attacks of acute tetany, which disappear spontaneously after a few weeks providing only ischemia of the parathyroids or relative parathyroid insufficiency after removal of a parathyroid adenoma is present. When there is severe damage to the parathyroids, latent tetany becomes chronic. Although much is known about the symptoms of chronic postoperative parathyroid insufficiency, very little is known about the epileptic seizure which occasionally arises as the only symptom. On the other hand, investigations on a large number of thyroidectomized patients in the last few years have shown that latent or partial parathyroid insufficiency without tetany can be demonstrated by means of suitable methods (p. 945) more frequently than expected. There is always a multitude of more or less severe functional and psychic symptoms. This clinical picture is often not recognized.

Idiopathic hypoparathyroidism (p. 899) is definitely a chronic disorder. It is seldom diagnosed in the newborn or infant. The first symptoms usually appear during the second decade. Attacks of spasm almost always occur in infantile idiopathic hypoparathyroidism and sometimes have a fatal outcome. Forms of the disorder with epileptic seizures as the only symptom are particularly common at this age. If the children survive, their mental and physical development is usually severely impaired.

Idiopathic hypoparathyroidism not uncommonly causes few symptoms and goes unnoticed in adults too. Tetany can remain latent without an acute attack ever occurring in spite of a very low serum calcium. This is probably due to a relative adaptation of the neuromuscular receptors to chronic hypocalcemia, and hypocalcemia is certainly not the only cause of tetany. Thus, mental (endocrine psychosyndrome) and dystrophic (cataract and osteoarticular syndrome) disorders are in the foreground. The question of the onset of the disorder arises in every newly diagnosed case of hypoparathyroidism in adulthood. Many years can pass between the onset of the first symptoms and the diagnosis. The diagnosis is often not made until adulthood although the case history shows that the first symptoms went as far back as infancy. The state of the teeth is a particularly good indicator of the time of onset. Some cases of idiopathic hypoparathyroidism may very well arise during adulthood; there is then no history of early symptoms. From post-mortem observations, it can be concluded that the vast majority of cases begin before the 16th year of life. The frequency declines in the 2nd and 3rd decade and increases again slightly in the 4th decade.

The diagnosis is even more difficult in advanced age. The advanced hypocalcemic cataract can no longer be differentiated from a senile cataract, and mental changes can be attributed to senility. Few cases of idiopathic hypoparathyroidism have been diagnosed for the first time between the 60th and 80th years of life.

It is important to realize that the course can be very variable. Clinical symptoms and biochemical findings can vary over long periods of time. Occasionally, serum calcium falls to a yearly minimum in spring following the dark winter months, indicating D-hypovitaminosis (p. 884). The cyclic variations in mineral

and bone metabolism in the female under the influence of estrogens suggest that estrogens also have some effect on the long-term course of hypoparathyroidism.

3. Diagnosis

The diagnosis is obvious if a hypocalcemic attack of tetany follows thyroid or parathyroid surgery. The clinical diagnosis can be difficult otherwise, due to the multiplicity of signs and symptoms, the varying severity of the hormone insufficiency, the varying duration of the disorder, and the wide variation in the sensitivity of the receptors to hypocalcemia. We have seen that age plays an important role in the symptomatology, e.g. in neurologic changes. Because of this, the clinical picture in the child differs greatly from that in the adult.

Diagnosis is particularly difficult when hyperthyroidism is superimposed on existing hypoparathyroidism. Hyperthyroidism causes a rise in calcium excretion in the urine and a lowered calcium level is sometimes normalized. Thus, hypoparathyroidism can be overshadowed clinically and biochemically by the development of hyperthyroidism. This is especially important for the choice of treatment of recurrent thyrotoxicosis.

It is easier to investigate suspect cases than to pick them out more or less by intuition from the patients in a practioner's consulting room or a hospital clinic. General features of hypoparathyroidism can be so variable and so confusing that the diagnosis is not made for years. The symptoms can simulate angina pectoris or other vasomotor circulatory disorders and even gastrointestinal disorders. It can also be similar to a psychoneurosis or even a psychosis. The doctor should therefore at least consider the possibility of hypoparathyroidism in all vague circulatory disorders, spastic illnesses of the gastrointestinal tract, nonspecific headaches, all neurotic and psychotic conditions, and in every case of laryngospasm or epilepsy. The condition must be suspected in every patient who has undergone thyroid surgery. Occasionally the ophthalmologist is the first to diagnose hypoparathyroidism on discovery of a tetanic cataract.

Laboratory investigations and functional tests are discussed on p. 940.

4. Differential Diagnosis

Difficulty in differential diagnosis can arise not only with cataract, cerebral calcifications, changes of the skin, hair and nails, dental changes and the osteoarticular syndrome, but with every physical symptom. Hypoparathyroidism is characterized by hypocalcemia, atypical behavior of the alkaline phosphatase, hypocalciuria, very low concentration of immunoreactive parathyroid hormone, and neurological symptoms.

a) Differential Diagnosis of Hypocalcemia

Hypocalcemia is one of the biochemical symptoms of D-hypovitaminosis due to malabsorption or other alimentary disorders. It is always accompanied by hypocalciuria (as is hypoparathyroidism), by hypophosphatemia (in contrast to hypoparathyroidism) and by a rachitic-osteomalacic osteopathy with elevated levels of alkaline phosphatase in the blood (in contrast to normal alkaline serum phosphate levels in hypoparathyroidism). Hypophosphatemia and the elevated serum phosphatase constitute the most important criteria for the differential diagnosis between hypocalcemia due to D-hypovitaminosis and that due to parathyroid insufficiency.

Hypocalcemia can, however, also have *renal causes*. In certain tubular nephropathies, the hypocalcemia is accompanied by hypophosphatemia and a rachitic-osteomalacic osteopathy with hypercalciuria (Albright-Lightwood syndrome) or with hyperphosphaturia (vitamin D-resistent rickets). Glomerular, azotemic renal insufficiency of very long duration can produce hyperphosphatemia and hypocalcemia as well as hypocalciuria and a mixed osteopathy (osteomalacia and ostitis fibrosa). In such cases, however, the disorders of calcium and phosphate metabolism are dominated by the symptoms of renal insufficiency. Tetanic symptoms due to hypocalcemia are almost always absent due to the coexisting acidosis (the reader is also referred to the section on secondary hyperparathyroidism). Hypocalcemia of entirely different etiologies is observed in acute pancreatitis and in certain forms of metastatic skeletal carcinomatosis (chronic leucopathia, carcinoma of prostate, breast and bronchus (HALL, 1966), and also in medullary carcinoma of the thyroid secreting calcitonin.

Concentration of Immunoreactive Parathyroid Hormone in the Serum (iPTH). iPTH is elevated in all other forms of hypocalcemia leading to secondary hyperparathyroidism. iPTH concentrations are elevated in pseudohypoparathyroidism, nutritional rickets of infancy, intestinal malabsorption, and renal insufficiency. iPTH may be undetectable in some normocalcemic, normal subjects, depending on the sensitivity of the radioimmunoassay used. However, in

hypocalcemia iPTH is usually elevated, and every form of secondary hyperparathyroidism can thus be clearly differentiated from hypoparathyroidism (REISS, 1968; ARNAUD, 1971; FISCHER, 1973).

b) Differential Diagnosis of the Neurologic Syndrome

We have seen that the clinical picture of hypoparathyroidism is dominated by tetany but that tetany with all its features of neuromuscular overexcitability also occurs in numerous other pathologic conditions. A distinction must be made between hypocalcemic and normocalcemic tetany. The most common causes of hypocalcemia are discussed above (p. 890). Tetany is observed in all these conditions, but is not a mandatory feature. It is absent in hypocalcemic nephropathies accompanied by acidosis.

Normocalcemic tetany is observed in different conditions which may not be related to calcium and phosphate metabolism and parathyroid function and in which there are no dystrophic changes of hypoparathyroidism. The most common cause of normocalcemic tetany is alkalosis arising after hyperventilation, prolonged vomiting, after oral and parenteral administration of alkaline salts (bicarbonate) and in primary aldosteronism (p. 333). The ionized calcium concentration is clearly decreased, whereas the total serum calcium, phosphorus, and phosphatase, and the urinary excretion of calcium are normal. Tetany following hyperventilation is encountered mainly in females and can present as acute tetany as well as less characteristic chronic disorders such as depression and paresthesia. Vegetative circulatory disorders (angina pectoris, extrasystoles, tachycardia) can also be a manifestation of hyperparathyroidism. The attack and the chronic state are both characterized by anxiety as the predominant symptom. The fear is psychic in nature and is maintained by the physical symptoms of tetany. Hyperventilation can provoke acute tetany and other chronic symptoms which complicate the diagnosis. Tetanic symptoms disappear when exhalation of carbon dioxide is interrupted by closing the mouth and nose or by breathing into a paper bag or inhaling carbon dioxide. The diagnosis is usually not difficult in most cases if the psychoneurotic element of the personality is taken into account.

Tetany is induced by a reduction in the concentration of ionized calcium, but this may not be the only cause.

So-called idiopathic tetany (not to be confused with idiopathic hypoparathyroidism) was first described during World War I, and the term is still used occasionally. It can be defined as an illness with symptoms of latent and manifest tetany in normocalcemic individuals of poor social standing. Current views class some of these cases under hypocalcemic tetany in D-hypovitaminosis and others as alkalotic tetany.

One form of tetany is caused indirectly by a rise in the level of serum phosphorus and a fall in the concentration of serum calcium and less frequently of magnesium.

Unless there is antagonism between serum calcium and serum phosphorus, hyperphosphatemia does not cause tetany.

Magnesium Deficiency. In man, a fall in the level of serum magnesium is observed in primary hyperparathyroidism after resection of an adenoma of the parathyroid. This is applicable particularly to cases of hyperparathyroidism with marked bone involvement, especially if vomiting occurs at the same time. Magnesium deficiency also occurs after infusions of large quantities of fluid devoid of magnesium, in chronic alcoholism, liver cirrhosis, malabsorption, intensive diuretic treatment with thiazides, tubular acidosis, renal insufficiency and primary hyperaldosteronism. Extreme magnesium deficiency presents, especially in children, as tremor, athetotic-choreiform movements, muscle weakness, giddiness, and irritability. Convulsions reminiscent of a grand-mal attack are dangerous but rarely fatal. Treatment of magnesium deficiency is discussed on p. 932.

c) Differential Diagnosis of Epilepsy

We have already seen that the grand-mal epileptic seizure, or occasionally petit-mal epilepsy, can be the presenting symptom of idiopathic hypoparathyroidism and less commonly of postoperative hypoparathyroidism. The symptomatology of this form of epilepsy completely hides that of genuine epilepsy. The EEG does not help in the differentiation. Relevant conclusions can be drawn only from the therapeutic test. Anticonvulsive treatment which inhibits true epilepsy has no effect on epilepsy associated with hypoparathyroidism, whereas treatment which corrects the hypocalcemia clinically cures parathyroid epilepsy as well as normalizing EEG findings. Although these cases are uncommon, they are of great practical importance. Clinical symptoms of latent tetany should be routinely searched for and serum calcium estimated whenever possible in epileptic institutions. The same applies to disorders of mental development, retardation and character

changes in children, and in adults, illnesses with vague psychological features and Parkinson's disease.

E. Idiopathic Hypoparathyroidism and Pseudohypoparathyroidism

B. COURVOISIER

1. Clinical Definition

a) Idiopathic Hypoparathyroidism (IHP)

This term embraces a chronic syndrome with the following clinical criteria:

1. biochemical syndrome: hypocalcemia, hyperphosphatemia, hypocalciuria;
2. tetany (latent or manifest);
3. dystrophies (facultative): cataract, cerebral calcifications, enamel hypoplasia and anomalies of dental formation, osteoarticular syndrome, dystrophies of skin, hair and nails (p. 894);
4. no history of surgery or ^{131}I therapy in the parathyroid region;
5. no other causes for the hypocalcemia, i.e. no D-hypovitaminosis, no intestinal malabsorption and no renal insufficiency.

IHP is an uncommon condition. About 200 cases have been described in the literature. Both sexes are equally affected. The first symptoms usually arise during late childhood or in adolescence. Many years can go past before the diagnosis is made.

In a review of 22 autopsied cases of IHP the parathyroids were atrophied, replaced by fatty tissue or not found (ZELTNER, 1969). Familial incidence has been observed in many cases, especially when the parents were consanguineous (BUCHS, 1955–1957; PEDEN, 1960; ZELTNER, 1969). These facts suggest that IHP is sometimes the result of a sex-linked or autosomal genetic defect with expression in early or late life. The etiology of sporadic IHP is obscure.

Autoimmune Endocrinopathies, Simultaneous Occurrence of Idiopathic Hypoparathyroidism with Addison's Disease and Pernicious Anemia. This syndrome is discussed in more detail in Chap. XVIII (p. 1009) (pluriglandular syndrome) where the coexistence of IHP with primary chronic adrenocortical insufficiency and of Hashimoto's thyroiditis with hypothyroidism are dealt with, together with BLIZZARD'S investigations demonstrating the presence of antibodies to parathyroid tissues in a large percentage of these cases. There is a familial incidence of IHP and moniliasis (SUTERKIN, 1943). Pernicious anamia is often found associated with IHP. In addition, in several cases, Addison's disease was found to be present and antibodies to the "intrinsic factor" and adrenocortical tissue were demonstrated. Pernicious anemia usually begins late in the course of the IHP.

b) Pseudohypoparathyroidism (PSHP)

ALBRIGHT coined this term (1942) for an illness which in addition to the 5 criteria mentioned is also characterized by the following 4 criteria:

6. dwarfism and round face;
7. brachydactylia;
8. subcutaneous calcifications;
9. negative Ellsworth test (no phosphaturia after intravenous injection of parathyroid extract) (p. 945).

In this Ellsworth test, the reaction of renal excretion of phosphate to an intravenous injection of a parathyroid extract is examined. In postoperative hypoparathyroidism, the test causes a marked rise in renal phosphate excretion which is normally diminished. ALBRIGHT demonstrated a similar reaction in idiopathic hypoparathyroidism, except in a few cases in which renal excretion of phosphate reacted only insufficiently or hardly at all to the injection of parathyroid hormone.

ALBRIGHT assumed that the last criterion in the list was due to primary renal resistance to parathyroid hormone (PTH). With this assumption in mind, be put forward the hypothesis that PSHP was not to be considered the expression of a hormone insufficiency, but much more as a failure of the receptor organs to respond to PTH. This interpretation was confirmed by his findings in parathyroid biopsies in three cases, one biopsy showing normal parathyroid tissue and two showing parathyroid hyperplasia.

MARTIN (1940) gave the first detailed description of this syndrome; all significant criteria are summarized here without any theory about the pathogenesis.

PSHP is also a rare disorder (less than 50 cases in the literature). It occurs more frequently in females than in males. The onset usually dates back to late childhood or adolescence, but the diagnosis is often made much later. Clinical symptoms coincide with the principal features of IHP. In addition there are numerous constitutional anomalies and subcutaneous calcifications. Usually the patient's appearance is conspicuous. Stature is small and stocky, the dwarfism being proportional or the extremities conspicuously short. The hands are short and broad. The face is round and the features quite expressionless.

Mild obesity is rarely observed. Oligophrenia is common. Bones of the hands and feet are disproportioned, the phalanges, particularly metacarpals and metatarsals 1, 4, and 5, being shortened due to impaired endochondral growth. The rest of the skeleton shows the same osteoarticular changes as are found in IHP. Calcifications in the soft tissues can occasionally be palpated as small, hard, movable and indolent subcutaneous nodules. Usually they are first detected on X-rays. They are found mainly on the extremities and are para-articular, subcutaneous, intratendinous or intramuscular.

The basal metabolic rate is frequently found to be reduced in PSHP. Recent investigations have not shown this to be due to primary hypothyroidism or to a peripheral resistance to thyroid hormones, but rather to insufficient secretion of thyrotropic hormone (WINNACKER, 1967; ZISMAN, 1969).

In PSHP the renal (and perhaps skeletal) resistance to PTH is the basic metabolic defect. The constitutional anomalies and the subcutaneous calcifications appear to be separate, unrelated inherited defects (FRAME, 1972). The PTH resistance is therefore independent of these abnormalities.

c) Brachymetacarpal Dwarfism without Hypocalcemia (Pseudopseudohypoparathyroidism after Albright)

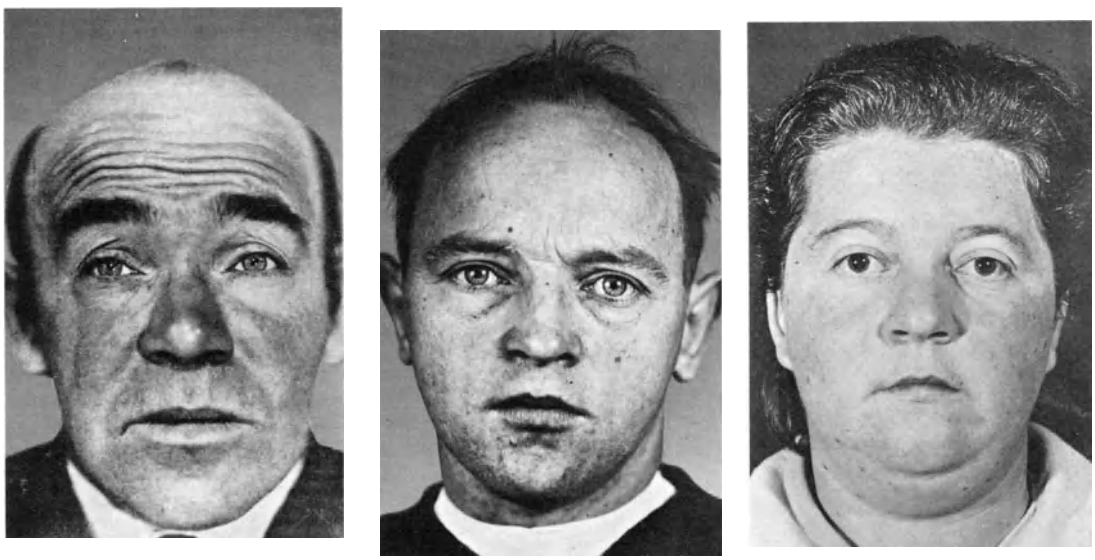
Examination of the families of patients with PSHP has revealed relatives with brachydactylia, round face and subcutaneous calcifications but no hypocalcemia. Furthermore, there are isolated cases as well as many familial cases where only constitutional anomalies and subcutaneous calcifications are found and no hypocalcemia or other symptoms of hypoparathyroidism. This syndrome was given the bizarre name of pseudopseudohypoparathyroidism by ALBRIGHT (1952). This term is questionable, and attempts have been made to replace it by other terms (dysplasia with calcification of soft tissues, Albright's dystrophy, brachymetacarpal dwarfism). It is now certain that the simultaneous occurrence of so many polymorphic dysplasias cannot produce a uniform picture, particularly since other types of malformations are found at the same time in the skin, skeleton, cardiovascular, urogenital and endocrine systems: anomalies of skin pigmentation, hypertrichosis, bone malformations, gonadal dysgenesia, pheochromocytomes etc. New dysplasias belonging to this group are continually being reported in the literature at the moment.

The occurrence of PSHP and brachymetacarpal dwarfism without hypocalcemia in the same family indicates that both disorders have a common genetic basis.

The discovery of constitutional abnormalities or subcutaneous calcifications without endocrine malfunction is of practical interest, since other members of the family could be affected by PSHP.

2. Pathogenesis

ALBRIGHT's hypothesis that PSHP is not a hormonal deficiency but a nonresponse of the



a b c

Fig. 21a–c. Three patients with round face and undifferentiated features characteristic of pseudohypoparathyroidism (a: from MARTIN, 1940; b: from SCHÜPBACH, 1949; c: a case from Zurich, a 33-year-old imbecile female patient)

receptors seems to be confirmed. In fact, three different forms of PSHP can be established (FRAME, 1972).

a) Renal and Skeletal Resistance to PTH

The absence of increased urinary phosphate excretion after PTH administration is considered a major diagnostic feature. The hormone is incapable of inhibiting phosphate tubular resorption. CHASE and AURBACH (1967, 1968) showed that the adenyl cyclase-3′-5′ cyclic AMP system in the tubular cells is activated by PTH and in normal subjects the hormone increases the urinary excretion of 3′-5′ cyclic AMP. In PSHP the renal tubular resistance to PTH is demonstrated by the absence of increased urinary phosphate and cyclic AMP excretion in response to parathyroid hormone administration (CHASE, 1969). Thus the primary fault in PSHP is the inability of the renal tubule cyclic AMP system to be activated by PTH. The consequence is increased phosphatemia that is partially responsible for the hypocalcemia. The extent to which the adenyl cyclase-cyclic AMP anomaly lowers the distal tubular calcium reabsorption which is normally stimulated by PTH has yet to be determined. Hypocalcemia increases PTH secretion: immunoreactive PTH concentrations in the serum are usually elevated (LEE, 1968; CHASE, 1969; FISCHER, 1973). Hypocalcemia together with clinical or X-ray and histologic evidence of hyperostosis, as in IHP, implies not only renal but also skeletal resistance to PTH. PTH normally activates the adenyl cyclase-cyclic AMP system in both kidney and bone (CHASE, 1969; FEDAK, 1969). However, a nonresponse of this kind has not yet been demonstrated in bone. One indirect argument for this case is the absence of increased amounts of hydroxyproline in the urine after PTH administration in PSHP in contrast to the rise in normal subjects and/or IHP (MCDONALD, 1972).

b) Skeletal Resistance to PTH with Normal Kidney Response

It is possible in some cases for only the skeletal adenyl cyclase-cyclic AMP system to be unresponsive to PTH, while activity in the kidney is normal (FRAME, 1972). This would explain the cases observed in which PSHP is associated with hyperostosis, hypocalcemia and a constant (SCHÜPBACH, 1949; COURVOISIER, 1963) or increased urinary phosphate response to PTH (FRAME, 1962). A change of this kind has also been observed in a case with clear-cell hyperplasia (FRAME, 1962).

c) Renal Resistance to PTH with Conserved Skeletal Receptivity

Several cases of biochemical hypoparathyroidism combined with renal resistance to PTH (absence of increased urinary phosphate and/or cyclic AMP excretion), as well as X-ray and histologic signs of osteitis fibrosa cystica have been reported in the literature (FRAME, 1972). This suggests that the renal resistance to PTH brings about hyperphosphatemia partially responsible for the hypocalcemia, thereby stimulating the parathyroids, as in group a). Some of these cases of secondary hyperparathyroidism were confirmed by radioimmunologic hormonal assay and/or histologic evidence of parathyroid hyperplasia. The osteitis fibrosa cystica is proof of the normal bone receptiveness to PTH. The mobilized bone calcium should correct the hypocalcemia, but it is possible that the calcemic effect is blocked or blunted by the hyperphosphatemia and also that there is a defect in osteocytic osteolysis (FRAME, 1972). As in the first group, the extent to which the adenyl cyclase-cyclic AMP anomaly lowers the distal tubular calcium reabsorption has yet to be established.

Finally these observations show that some patients have constitutional abnormalities, as described by ALBRIGHT, while others have a normal phenotype.

Other hypotheses have been suggested to explain PSHP: impaired biosynthesis of PTH, resulting in the *secretion of a biologically inactive hormone*, has been postulated, as has the presence of a *substance inhibiting PTH* in the blood or tissues (MANN, 1962). The detection of a very high concentration of calcitonin in the thyroid gland of some patients with PSHP (50–100-fold increase) (ALIAPOULIOS, 1966; TASHJIAN, 1966) gave rise to the speculation that the hypocalcemia is due to an *excessive secretion of calcitonin*. However, the concentration of calcitonin was found to be increased only in the thyroid gland and not in the peripheral blood of these patients. An increase in the concentration of calcitonin in the thyroid does not necessarily reflect an increased secretion but can also result from a diminished secretion. The ineffectiveness of total thyroidectomy on the plasma level of calcium in these patients contradicts the view that hypercalcitonism of thyroid origin is the cause of this syndrome (SUH, 1969).

3. Hereditary Factors

The condition can also be looked upon as a heredopathia (familial incidence and occurrence

in identical twins). Inheritance seems to occur through a dominant gene. Transmission is irregular and the localization of the gene is unknown. The women : men ratio of 2 : 1 suggests that the disorder is connected with the X chromosome (MANN, 1962). ELRIK and ALBRIGHT (1950) postulated some time ago that a three-fold genetic anomaly might be present embracing the following 3 groups of symptoms: 1. failure of the receptor organs to respond to PTH, resulting in the same changes as are found in IHP; 2. constitutional anomalies, a type of dyschondroplasia, particularly of the metacarpals and metatarsals; 3. tendency to development of subcutaneous calcifications.

F. Treatment of Hypoparathyroidism

B. COURVOISIER

a) General Facts

Treatment of parathyroid insufficiency involves 2 types of drugs:

1. calcium salts for intravenous and oral use;
2. the steroids, vitamin D and dihydrotachysterol.

Parathyroid hormone cannot be used therapeutically in the form now available. The commercially available extract induces the formation of antibodies and leads to "hormone resistance". Antibodies to bovine parathyroid hormone have recently been demonstrated for the first time in man.

There are two types of vitamin D; vitamin D_2 is of vegetable origin and vitamin D_3 of animal origin.

Vitamin D_2, or calciferol, is derived from egosterol, which is contained in ergot and barm, by means of a photochemical reaction under the influence of ultraviolet light.

Vitamin D_3, or cholecalciferol, is the natural vitamin D of animal origin and is derived from 7-dehydrocholesterol. In man, endogenous vitamin D_3 is synthesized in the skin under the action of ultraviolet light. Exogenous vitamin D_3 is obtained from food, being found predominantly in certain fish oils, and in smaller amounts in milk, butter and egg yolk. Synthetic vitamin D_3, which is identical to the naturally occurring substance, has been in therapeutic use for the past few years. The recently detected more active metabolites 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol (p. 883) are not available for therapeutic purposes and probable will not be, whereas therapeutic interesting analogues may be found.

Dihydrotachysterol is obtained by irradiating ergosterol and is used for the treatment of hypocalcemia as AT 10 (antitetanic factor 10).

Chemically pure vitamin D_2 and D_3 are, however, quite as suitable as dihydrotachysterol for the treatment of hypocalcemic states. JESSERER compared both vitamins with dihydrotachysterol and detected only insignificant pharmacological differences: aqueous solutions of all 3 substances are equally effective when given by i.m. or i.v. injection. An oily solution of vitamin D given by the i.m. route is effective, whereas an oily solution of dihydrotachysterol has no effect. In contrast, when the preparations are given orally dihydrotachysterol is more potent than vitamin D. All preparations can be used for the treatment of hypocalcemic states. It is important to know that the individual reactions to vitamin D and dihydrotachysterol vary and can do so in the same person at different times. The correct dose must therefore be chosen with care in such a case. The biological half-life of vitamin D_2 and D_3 is some weeks or even months, and vitamin-D intoxication is a serious problem in the treatment of hypoparathyroidism. According to KAYE, the biological half-life of dihydrotachysterol is shorter and may therefore be more useful in the treatment of hypoparathyroidism than vitamin D.

b) Treatment of Acute Tetany

Acute tetany is most commonly observed in postoperative hypoparathyroidism, the first attack usually arising as soon as a few hours after thyroidectomy or removal of a parathyroid adenoma. Acute tetanic attacks are, however, also seen in idiopathic hypoparathyroidism. Treatment consists of an intravenous injection of calcium salt: an injection of 20 ml 10% solution of calcium gluconolactonate (= 180 mg calcium) given slowly intravenously is most suitable. Blood for the estimation of serum calcium and phosphate must be taken before the injection is given. It is not necessary to wait for the results of these estimations since treatment with calcium is harmless and produces no ill effects in normocalcemic tetany. If the patient's response to the first injection is inadequate or only temporary the injection should be repeated once or twice hourly for the next few hours until the result of the serum calcium estimation is available. It must be stressed that the effect of intravenous injection of calcium is only short due to rapid fixation of calcium in the skeleton and rapid excretion through the kidneys. Because of this it may be necessary to start an intravenous infusion with calcium (15 mg Ca^{++}/kg body weight in

500 ml physiological saline). As soon as the diagnosis of hypocalcemic tetany is confirmed, and depending on the severity of the hypocalcemia, treatment is supplemented by a steroid promoting a rise in serum calcium. Crystalline vitamin D_3 in an aqueous solution is most potent and has the fastest onset of action. Depending on the serum calcium level, which is often very low (6 mg/100 ml and even lower), 4–6 ampoules (1 ampoule contains 7.5 mg = 300000 units)) are given. The full effect of this treatment cannot be expected until after 1–3 days, in spite of the intravenous route of administration. Meanwhile, an uninterrupted infusion with calcium is maintained. Repeated serum calcium estimations are essential for an adequate dosage. Sedation with a barbiturate can also be useful during this phase. Phenothiazine compounds of any sort must be avoided, since as was seen above (p. 893) extrapyramidal features of hypoparathyroidism can occasionally be accentuated or severe neurodysleptic attacks induced. When tetanic symptoms do not cease even after normalization of serum calcium in spite of continuous treatment and high doses of vitamin D, the possibility of magnesium deficiency must be considered, particularly in postoperative hypoparathyroidism following the removal of a parathyroid adenoma, though this does occasionally happen with other forms of hypocalcemic tetany. After serum magnesium has been estimated, substitution therapy must be started at once, following the schedule described in the chapter dealing with the postoperative course and treatment of primary hyperparathyroidism (p. 932). The hypocalcemia is generally corrected within 1–8 days following intravenous injection of vitamin D. If correction fails to occur, a further 7.5 mg vitamin D_3 must be given intravenously, and if repeated administrations of vitamin D are still required after a few weeks, long-term treatment adhering to the principles given below must be introduced. Not uncommonly, however, the serum calcium level becomes normal spontaneously after a few weeks or, more infrequently, months in postoperative hypoparathyroidism due to compensatory hyperplasia of the still intact parathyroids. The patient can then be considered cured providing estimations of serum calcium every 3–6 months for the following 2 years give favorable results.

c) Treatment of Chronic Postoperative and Idiopathic Parathyroid Insufficiency

Vitamin D and dihyrotachysterol given by the oral route are the drugs of choice. The most important aims of treatment are normalization of serum calcium at a level between 9 and 11 mg% and the prevention of hypercalciuria. The dangers of hypercalcemia and hypercalciuria (nephrolithiasis, nephrocalcinosis etc., p. 918) and of hypocalcemia (tetany, neurologic changes, cataract and other changes, p. 893) have been discussed in detail above. Serum calcium can vary spontaneously over several months, rising without warning to normal values or falling to very low levels.

Thus, with the exception of cases of transient postoperative parathyroid insufficiency, hypoparathyroidism can never be considered cured. Patients need treatment for the rest of their lives, serum calcium must be checked regularly and normalized with vitamin D when necessary. No fixed daily dose can be given due to the marked individual variation. The dosage must be adjusted continually from estimations of the serum calcium. Serum calcium and the 24-hour excretion of calcium in the urine must be measured once to twice monthly at first, and later every 3 to 6 months. It must be remembered that the variations in the dosage in individual cases can be associated with the facts that a lower dose is needed during the sunny summer and autumn months than in winter and spring, and that vitamin-D requirements are increased by mobilization and decreased by immobilization. Vitamin-D requirements are increased by hypothyroidism and decreased by hyperthyroidism. *Treatment must be discontinued when serum calcium exceeds 10.0 mg/100 ml and urinary excretion of calcium is more than 300 mg in 24 hours.*

The dosage depends of the seriousness of the disorder and should be adapted to each individual patient. The following dosage schedule for vitamin D or dihydrotachysterol will serve as a guide:

Initial dose: 1–2 mg daily for several days.
Average maintenance dose: 0.5–1.0 mg.

Vitamin D and dihydrotachysterol have a cumulative action. For this reason treatment can also be intermittent: 1.0–3.0 mg twice weekly.

The diet for patients with chronic hypoparathyroidism should contain adequate amounts of calcium. The maintenance doses of vitamin D and dihydrotachysterol can be reduced by regular administration of calcium (1.0 g calcium daily). Postoperative hypoparathyroidism is occasionally associated with hypothyroidism. Since thyroid hormones cause calcium mobilization, i.e. raise a reduced serum calcium level and increase urinary excretion of calcium, substitution therapy for thyroid insufficiency is strongly indicated. Resistance to vitamin D and dihydrotachysterol has occasionally been observed in postoperative hypo-

parathyroidism. In one such case, it was observed that pregnancy terminated the vitamin-D resistance. In another case, the normal response to vitamin D, which had previously been present, was restored by giving 25 mg cortisol orally, twice daily.

G. Primary Hyperparathyroidism

M. WERNLY

1. Definition

Primary hyperparathyroidism is caused by excessive synthesis and release of parathyroid hormone in the absence of an elevated hormone requirement of the organism. The most common morphological findings in the parathyroids are 1. the solitary parathyroid adenoma, and less commonly, 2. multiple parathyroid adenomas or 3. multiple parathyroid adenomas associated with the syndrome of the endocrine adenomatosis ("chief-cell hyperplasia"); 4. primary hyperplasia of the water-clear cells and 5. carcinoma of the parathyroid gland may be responsible.

Secondary and tertiary hyperthyroidism are discussed below (p. 934, 939).

2. Etiology, Incidence and Frequency

Etiology. With the exception of endocrine adenomatosis, which is most probably due to a gene mutation, nothing is known about the etiology of primary hyperparathyroidism. Adenomas of the parathyroids are independent of the functional state of the pituitary gland. Contrary to earlier suspicions, the serum phosphorus does not directly influence the formation of an adenoma. However, an increase in the serum phosphorus concentration always leads to hypocalcemia and thus to an increased parathyroid hormone secretion.

The term "tertiary hyperparathyroidism" describes a pathogenic form of primary hyperparathyroidism. It has arisen from a small number of clinical cases associated with the development of parathyroid adenoma causing hypercalcemia from hypocalcemic secondary hyperparathyroidism. This rare occurrence has been observed so far in a few cases of intestinal malabsorption and chronic renal failure, which presented clinically for years as secondary hyperparathyroidism with hypocalcemia. During the clinical course there was a slow change to normo- and hypercalcemia, and at the same time the clinical picture of secondary hyperparathyroidism was replaced by that of primary hyperparathyroidism. The morphological changes causing this condition are found at operation to be a diffuse or microadenomatous hyperplasia of all 4 parathyroids, sometimes associated with a well-defined large parathyroid adenoma. The removal of this adenoma cures the primary, alias tertiary, hyperparathyroidism in the classic way. In addition to the usual course of development of the autonomic adenoma, there is thus a less common type of development of a reactive adenoma, in which the adenoma is preceded by secondary hyperplasia of the parathyroid glands due to hypocalcemia or hypomagnesemia. Tertiary hyperparathyroidism is discussed on p. 939.

It is not known whether there is any etiological or pathogenic connection with intercurrent sarcoidosis or gout.

Most parathyroid adenomas develop for no apparent reason. However, in a few cases, hyperplasia of the parathyroids related to renal or some other disease may precede the adenoma. The parathyroid adenoma in endocrine adenomatosis is most probably due to a genetic mutation (WERNER).

Incidence. In adulthood, primary hyperparathyroidism due to a parathyroid adenoma is at least twice as frequent in the female as in the male. There is no sex-linked difference in incidence before puberty or for the rare carcinoma of the parathyroid glands. Nor is it yet certain whether primary hyperplasia of all four glands is also more common in the female sex. Primary hyperparathyroidism is uncommon before the 2nd year of life. The 20–40 age group accounts for 30% of cases, and the 40–60 age group for 50%.

Frequency. Primary hyperparathyroidism used to be considered a rare disease, but the number of cases diagnosed is increasing continually with growing medical interest and the possibility of measuring parathyroid hormone in the blood directly by radioimmunoassay. The knowledge that 5% of all patients with nephrolithiasis suffer from primary hyperparathyroidism has contributed to the early diagnosis of cases of hyperparathyroidism. The true frequency of primary hyperparathyroidism, however, is deduced from the routine estimation of the serum calcium in a series of subjects chosen at random. Thus, BOONSTRA found 31 cases of hyperparathyroidism confirmed at operation in 26000 hospital admissions (8 presented clinically as nephrolithiasis, 13 had gastrointestinal symptoms, 17 suffered from fatigue to the point of exhaustion, 3 patients had mental depression, none had skeletal involvement). Thus there was at least one clinically unrecognized case of

primary hyperparathyroidism in 850 hospital patients, corresponding to a frequency of 1.2‰. However, it must be borne in mind that these figures for the frequency relate to patients admitted to hospital, who have some significant symptoms of disease, and not to a healthy population. The percentage incidence is considerably higher if parathyroid hormone concentrations are systematically measured even in normocalcemic subjects (PURNELL, 1971).

3. Pathologic Anatomy and Histology

A histologist experienced in work on the parathyroid glands should be present at every operation on the parathyroids. He should examine all the pieces of tissue excised by the surgeon throughout the operation and should identify them as being parathyroid tissue or tissue from other cervical organs. He should also make the differential diagnosis between the normal, hyperplastic, adenomatous or carcinomatous parathyroid tissue.

a) Frequency of the Various Findings in the Parathyroids in Primary Hyperparathyroidism

These data have been derived from four series of statistics (Table 4). The differences between one series and the other are due to the various ways of interpreting the histological findings by the different authors.

b) Solitary and Multiple Adenomas

Adenomas can be little larger than the normal glands but can also reach the size of an almond, of a cherry, or of a hen's egg. The weight varies between 25 mg and 100 g. The solitary adenoma does not differ macroscopically or microscopically from multiple adenomas. The adenomas are somewhat darker than the normal gland, which often has a pronounced yellow fatty appearance, and may be red-brown to red-yellow in color. In comparison with thyroid tissue, the parathyroids appear brighter and pink. Anatomists and surgeons also consider the parathyroids have a characteristic consistency, which is softer than that of the thyroid tissue, or of a lymph node, but considerably firmer than that of fat or thymic tissue. Every parathyroid adenoma possesses a clearly recognizable capsule. This capsule is only exceptionally penetrated by one or two pseudopodialike parenchymal processes. When they reach a certain size, most adenomas show a well-differentiated vascular stem, which is of great importance to the surgeon when the adenoma is situated in the mediastinum (p. 846). The possibility of a parathyroid adenoma within the thyroid must be taken into account (p. 931).

The sectioned surface is usually slightly darker than the outer surface. During sectioning, one or several cysts with bright yellow or hemorrhagic contents are sometimes opened. Adenomas with a completely regular appearance on the outside sometimes show a large number of microadenomas on being sectioned.

The histological preparations of parathyroid adenomas involving only a part of the glandular parenchyma show a globular, oval form. Their tendency to expand causes compression of the surrounding tissue. In medium-sized adenomas the compressed part of the normal parathyroid tissue shows up as a narrow stripe. Usually no more normal parathyroid tissue is found in the presence of larger adenomas. The presence of a stripe of normal parathyroid tissue is important for the histological diagnosis during an operation. Some fat cells are usually also

Table 4. The frequency given as a percentage of the different findings in the parathyroid glands in primary hyperparathyroidism

	Mayo Clinic Rochester (BLACK) up to Dec. 1959	Massachusetts General Hospital Boston (ROTH) up to Aug. 1959	Armed Forces Institute Washington (ROTH) up to Aug. 1959	Karolinska Syukhuset Stockholm (HELLSTRÖM) up to 1960
Solitary adenoma	89.1%	79 %	83.1%	84.8%
Multiple adenomas	2.9%	4.7%	2.8%	2.2%
Multiple parathyroid adenomas in endocrine adenomatosis	2.6%	–	–	–
Primary hyperplasia of the chief cells	–	6.7%	5.6%	2.9%
Primary hyperplasia of the water-clear cells	4.9%	6 %	2.8%	9.4%
Carcinoma	0.5%	3.6%	5.7%	0.7%
Number of cases	385	352	142	138

recognized in the stripe of normal parathyroid tissue, whereas fat cells are extremely rare in the adenoma. The behavior of the fat cells is also of diagnostic importance and is discussed on p.

The adenomas of primary hyperparathyroidism are composed of small and large chief cells, water-clear cells of all sizes, or of atypical oxyphil cells which are often larger and brighter than the usual oxyphil cells. As a rule, however, an adenoma contains not only one cell type but several. One cell type usually dominates the picture. The parenchymal cells of a functioning adenoma are analogous to normal cells or similar but never quite the same as those in secondary hyperplastic glands. They differ from normal cells in the following cytological properties: peculiar color tinges of the protoplasm with the usual methods of staining, which are not observed in normal cells, tendency to syncytial arrangement of the cell types; abnormally large nuclei of bizarre form (Fig. 22), numerous amitoses (50 nuclei or more in a narrow syncytial space). True and atypical mitoses are never found in this condition, in contrast to carcinomas of the parathyroids.

Parathyroid adenomas often show signs of secondary degeneration, which is probably due to the enormous cellular proliferation without the simultaneous formation of an efficient vascular network. This results in hypoxemic necrosis, hemorrhage and focal softening with cystic formation. Even subtotal or total necrosis of the adenoma sometimes occurs, in which case there may be a spontaneous clinical cure of the hyperparathyroidism. There are also cases of corresponding clinical observations with the typical "postoperative" hypocalcemic tetany (HOWARD, 1953; JOHNSTON, 1961).

The lipoid adenoma is considered as a parathyroid adenoma presenting clinically as primary hyperparathyroidism. Abundant, edematous fibrous tissue stroma with numerous fat cells is found between the chief cells, which are arranged in loose strands (OBER, 1958; ABUL-HAJ, 1962). A similar picture is occasionally found in a part of the gland in the presence of a classic parathyroid adenoma. Cystic parathyroid adenomas are discussed on p. 847 and p. 924.

Not every parathyroid adenoma is associated with primary hyperparathyroidism. There are adenomas which are not active, including predominantly the pure oxyphil adenomas seen in elderly persons (p. 847). They must be strictly differentiated from the active oxyphil adenomas in primary hyperparathyroidism, which are predominantly or exclusively composed of atypical oxyphil cells (p. 906). The fundamental difference between the functionless adenoma and the functioning oxyphil adenoma has recently been confirmed by electron microscope studies which showed that the cellular organelles of the oxyphil cells of the former adenoma type are inactive, while those of the second adenoma type are hyperactive (p. 847). Differentiation on histological grounds only can be difficult.

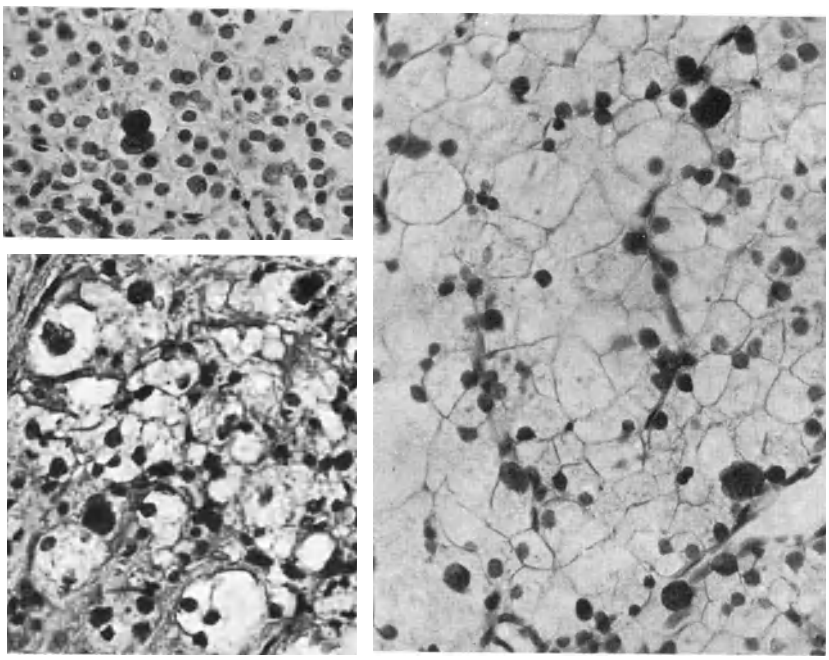


Fig. 22. Primary hyperparathyroidism. Abnormaly large nuclei with atypical form in three benign adenomas

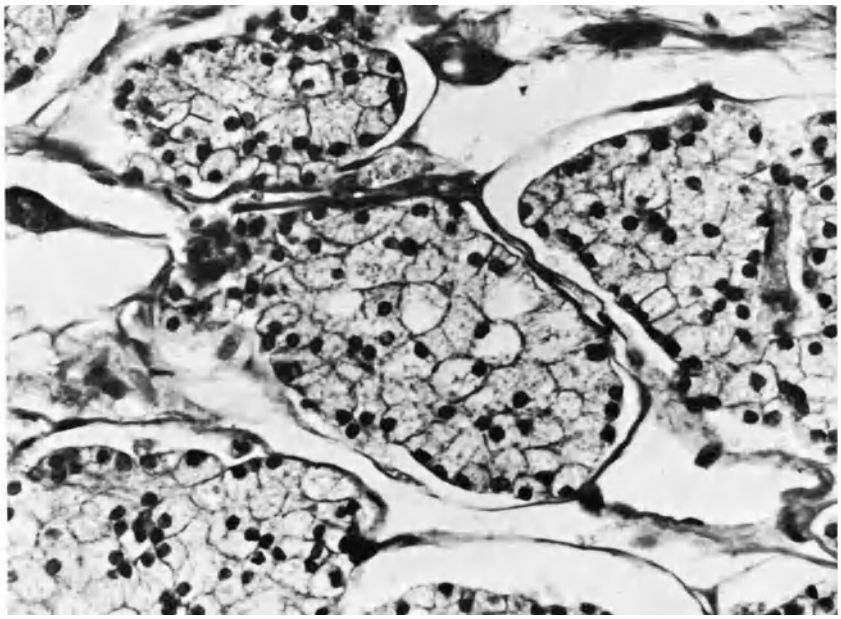


Fig. 23. Primary hyperplasia of water-clear cells in primary hyperparathyroidism

Recently, BLACK (1961) and RAMSDELL (1960) have also described parathyroid adenomas discovered incidentally during operations on the thyroid. These adenomas do not differ at all, morphologically, from functioning parathyroid adenomas. The subsequent, very precise clinical investigation revealed no clinical symptoms of hyperparathyroidism in most of these patients. In a few cases, there was hypercalcemia, or borderline hypercalcemia. There was no hypercalcemia in 7 of 21 cases. Whereas the cases of clinically silent hypercalcemia were assessed with no particular difficulty (chemical hyperparathyroidism without clinical symptoms, p. 911), assessment of normocalcemic cases is possible only with the aid of a sensitive radioimmunoassay for parathyroid hormone in the blood (chemically and clinically silent parathyroid adenomas are discussed on p. 911).

c) Primary Hyperplasia of the Water-Clear Cells

This condition is observed in one in 20 cases of primary hyperparathyroidism, and is characterized by a pronounced increase in the volume of all 4 glands. The weight of two or three of the 4 glands is usually over 10 g. One or two glands sometimes retain the normal weight. According to BLACK the total weight of all 4 glands is 760 mg–132 g, and according to ROTH 3–60 g. In contrast to the parathyroids in normal subjects, the upper glands are usually

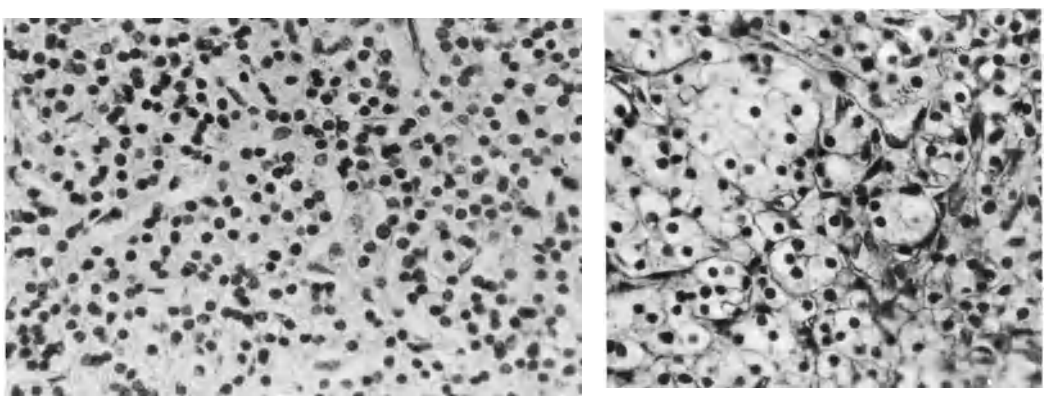


Fig. 24. Secondary hyperplasia of water-clear cells in secondary hyperparathyroidism. Left: chronic nephritis; right: osteomalacia

larger than the lower glands. The surface is very irregular in comparison with that of an adenoma, and numerous lobes and recesses as well as pseudopodia-like processes are seen. In spite of this the capsule is thin and transparent, and is easily separable from the surrounding tissue. The color is often a striking chocolate brown. These special properties of the surface and the color are only recognizable when the glands are grossly enlarged.

All 4 glands contain only one cell type, very large water-clear cells (Fig. 23). Even if one gland has retained its normal size, it consists entirely of these cells. Chief cells, small water-clear cells or oxyphil cells are only rarely discovered even with the most thorough inspection of the preparation. A stripe of normal parathyroid tissue is never found. Irregular parenchymal processes of the water-clear cells are not uncommonly seen in histological preparations, extending from the surface of the gland into the surrounding adipose tissue. Since a water-clear solitary adenoma can give a histological picture compatible with these findings, the diagnosis of primary hyperplasia of the water-clear cells is possible only in the presence of evenly distributed hyperplasia of the water-clear cells in all 4 glands.

The differentiation of this characteristic picture from that of secondary hyperplasia of all four glands in secondary hyperparathyroidism, as encountered in cases of chronic renal insufficiency or intestinal malabsorption, is of practical importance. The reader is referred to p. 934 for a discussion of the histology of this condition. The finding that the entire parenchyma is composed exclusively of large, water-clear cells is the decisive feature of the picture of primary hyperplasia of the water-clear cells.

The surgeon performing a subtotal resection of the total tissue of the gland, leaving a small, well-vascularized remnant of 50–100 mg, can often suspect primary hyperplasia of the water-clear cells from the chocolate brown color alone, but the histological findings in all 4 glands are essential for a definitive diagnosis. Difficulties can arise when 2 greatly enlarged glands on one side merge into one unit, as the surgeon cannot be quite certain whether he has found all 4 glands, and cannot carry out the intended subtotal resection.

d) Parathyroid Adenomas in Endocrine Adenomatosis

Multiple adenomas of the parathyroids are a form of endocrine adenomatosis (adenomas of the parathyroids, adenohypophysis, islet cells of the pancreas, adrenal cortex and medulla). They cause primary hyperparathyroidism about as frequently as hyperplasia of the water-clear cells. The adenomas of the parathyroids are always multiple in this syndrome, and in most cases every parathyroid gland reveals several small adenomas. Some of the cases recognized so far have been described as "primary hyperplasia of the chief cells" in the literature (p. 909). The frequent familial occurrence is probably an expression of a genetic mutation, and is characteristic of endocrine adenomatosis (Chap. XVIII, p. 1007).

All the parathyroids are considerably enlarged. ROTH gives the total weight of all 4 glands as 1–25 g. The upper glands are usually more enlarged than the lower. On several occasions one of the lower glands has proved to be situated in the mediastinum. The surface of the gland is irregular, and usually devoid of pseudopodia-like processes. The color is reddish-brown and not chocolate brown as in primary hyperplasia of the water-clear cells. A structure consisting of small nodules is macroscopically visible. The histology shows either a solitary chief-cell adenoma or microadenomatous hyperplasia of the chief cells. The size of these chief cells varies from field to field or from one microadenoma to the other. Their size, however, usually remains within normal limits. Fields with small water-clear cells are rarely found, and fields with larger water-clear cells are even rarer. In two cases, the glandular parenchyma was composed almost entirely of small and large clear oxyphil cells. Giant nuclei and amitoses are more uncommon in microadenomatous hyperplasia than in a solitary adenoma. A stripe of normal parathyroid tissue has never been observed. Fat cells are occasionally scattered in individual areas.

The picture described here is very similar to that of microadenomatous hyperplasia of secondary hyperparathyroidism (p. 934). Sometimes it is even impossible to differentiate between the two conditions from the histological picture of a single gland or of a glandular biopsy. In such cases, only the total clinical findings together with the histological findings of all 4 glands can be of any further help.

The following factors must be taken into account during surgical treatment: in every case of primary hyperparathyroidism in which clinical or histological examination suggests an endocrine adenomatosis all 4 glands should be very carefully examined during the operation. As a rule three glands are totally removed, and 30–50 mg of the smallest gland left behind. BLACK advocates the precaution of marking the residual parathyroid with a black silk

suture, so that in case of recurrence, which is not uncommon, the gland can be easily identified. The total excision of all four glands is the treatment of choice in theory, but this procedure will be permissible only when a substitution therapy better than the present method becomes available.

e) Primary Hyperplasia of the Chief Cells

The morphological findings in the parathyroids in this disorder are very similar to those in endocrine adenomatosis (p. 908). There are different opinions about whether the term hyperplasia or adenomatosis should be chosen. We prefer adenomatosis for of the following reasons: changes in the islet cells of the pancreas, in the adenohypophysis, and in the adrenal cortex are all termed adenoma or multiple adenomatosis and the same term should therefore be applied to changes in the parathyroids. However, the term "primary hyperplasia of the chief cells" has now become so well-established that it can no longer be excluded from the clinical and patho-anatomical terminology.

Most cases of primary hyperplasia of the chief cells or multiple parathyroid adenomas have been observed and described in association with endocrine adenomatosis. In a few cases, however, multiple adenomas of all four parathyroids have been the only findings, with no clinical or morphological evidence of involvement of the pituitary gland, the islet cells, or the adrenals. It is striking that there is an increased familial incidence in these cases (in 14 of 30 cases according to CUTLER), as there is in endocrine adenomatosis. Familial frequency, however, is one of the characteristic features of the syndrome of endocrine adenomatosis, and this therefore indicates that isolated, multiple parathyroid adenomatosis, or isolated primary hyperplasia of the chief cells, should also be included in the syndrome of endocrine adenomatosis.

f) Carcinoma of the Parathyroid

This is extremely rare. Many authors in the past, however, repeatedly referred to carcinomas of the thyroid gland as parathyroid carcinomas when the cellular picture of the tumor was similar to that of the parathyroids. However, the symptoms of hyperparathyroidism were absent, and the classification of such tumors as parathyroid tumors was not confirmed.

A few cases of parathyroid carcinomas have nonetheless been reported. They all showed clinical and biochemical features of primary hyperparathyroidism. The tumor in the parathyroids shows local malignant properties with infiltrative growth into the surrounding tissues, namely into the capsule of the adenoma, the thyroid gland, the large blood vessels, the muscles and the esophagus. Metastases occur frequently and are found in the regional lymph nodes, the lungs, liver, bones, and spleen. During surgery, the strong adherence of the tumor to the surrounding tissues is striking, and in direct contrast to the situation in cases of benign adenomas. The histological findings are less definitive. In most but not all cases (v. ALBERTINI), mitoses which are sometimes atypical are recognized, as well as distinct tumor invasion into the surrounding tissue. Amitosis and capsular invasion by themselves, on the other hand, cannot be interpreted as signs of malignancy, since they have also been observed in benign adenomas and in primary hyperplasias. The existence of endocrine-active metastases and recurrence of the tumor after surgical removal are the only features which can be considered criteria of malignancy.

The theoretical question of the existence of endocrine-inactive carcinomas of the parathyroids cannot yet be answered. It is not possible to make a diagnosis from the clinical features and the findings in the blood, and the local findings do not allow a distinction from the carcinoma of the thyroid gland. There is an unspoken rule that only tumors related to primary hyperparathyroidism by the clinical features and the findings in the blood are described as carcinomas of the parathyroids.

Carcinoma of the parathyroids has a typical clinical course. Hypercalcemia with more than 14 mg/100 ml is usually present.

Excision of the carcinoma results in the disappearance of all the symptoms of the disease but the cure is not lasting. There is always the possibility of a recurrence of the clinical symptoms and the pathologic findings in the blood, which can be associated with the formation of a recurrent tumor locally or with metastases. A second radical operation sometimes produces a cure lasting some months, and occasionally years. Finally, the terminal recurrence appears with the fully developed picture of hyperparathyroidism with all the clinical and biochemical findings. Death is due to renal insufficiency (nephrocalcinosis, renal vein thrombosis, renal hypertension and renal failure) or pancreatitis. An intercurrent illness can also have a fatal outcome. Autopsy reveals metastases of the carcinoma of the parathyroids in one of the organs mentioned. Carcinoma of the parathyroids and its metastases are resistent to radiotherapy.

4. Pathophysiology

The parathyroids are not controlled by any other known endocrine organ, such as the pituitary, in normal or pathologic conditions. A fall in the serum calcium and/or magnesium levels in the serum are the recognized stimuli for the secretory activity of the parathyroids. Conversely, their most important function is to regulate the concentration of calcium in the serum (p. 869, p. 889).

The clinical symptoms of primary hyperparathyroidism are entirely attributable to the autonomous elevation of the parathyroid hormone activity. The following changes have been observed in primary hyperparathyroidism due to the action of the parathyroid hormone at the three sites – the bones, the kidneys and the intestine.

1. Increased activity of the osteocytes, osteoblasts, and osteoclasts throughout the skeleton. In contrast to the normal physiological situation, the osteocytic osteolysis and the osteoclastic resorption dominate bone formation in primary hyperparathyroidism. This leads to an increased release of calcium and phosphorus into the extracellular space and into the serum. An elevated turnover of calcium and phosphorus is seen in the skeleton. This can be domonstrated by kinetic methods. Only a few patients, however, lose such large amounts of bone minerals that clinically recognizable osteopathy results. In most cases, the skeletal involvement is termed generalized osteitis fibrosa cystica (v. RECKLINGHAUSEN), while in a smaller number of cases there is a mixed osteopathy, consisting of generalized osteitis fibrosa cystica and osteomalacia.

2. Elevation of the phosphate clearance and reduction of the calcium clearance. The increased amounts of phosphate obtained from bone resorption are eliminated from the organism via the elevated renal clearance of phosphorus. Serum phosphorus remains normal or tends to fall. The reduced renal clearance of calcium favors hypercalcemia, as do the increased amounts of calcium released by destruction of the bones. Thus, hypercalcemia, normo- or hypophosphatemia, hypercalciuria and hyperphosphaturia are the four mandatory symptoms of primary haperparathyroidism (p. 924).

3. Increase in intestinal absorption of calcium. A third, intestinal hypercalcemic factor joins the osseous and renal factors producing hypercalcemia. More calcium is excreted by the kidneys than by the intestine. In healthy subjects on a normal diet, 80% of the calcium is excreted in the feces and 20% through the kidneys. In primary hyperparathyroidism the intestinal excretion falls, sometimes to 10%, with an increase of the renal fraction to 90%.

In addition to the changes in serum calcium and phosphorus levels, serum citrate may rise above 3.5 mg/100 ml in primary hyperparathyroidism, while the magnesium in the urine can be increased and the serum magnesium normal or slightly decreased (SUTTON, 1970). In some cases, the magnesium balance is negative.

Neuromuscular excitability is suppressed in hypercalcemia and increases in hypocalcemia, which accounts for the hypotonia of the striated muscles with asthenia and the atonic constipation found in hypercalcemic patients.

Extreme hypercalcemia as observed in acute hyperparathyroidism leads to polyuria, vomiting, dehydration and renal insufficiency with hyperphosphatemia. Metastatic calcification in numerous organs arises when the serum calcium and phosphorus are greatly increased at the same time (cf. acute hyperparathyroidism, p. 923). The development and assessment of additional indirect results of hypercalcemia (gastric ulcer, pancreatitis, mental and neurologic changes) are discussed in the clinical section.

Hypercalciuria related to or independent of hypercalcemia is the cause of the nephrolithiasis which occurs frequently in primary hyperparathyroidism. Nephrocalcinosis is seen predominantly but not exclusively in severe hypercalcemia of long duration. The diminished renal excretion of hydrogen ions with an alkaline urine in the presence of hypercalcemia has an additional effect on the nephrocalcinosis and the nephrolithiasis. The nephrocalcinosis can lead to a progressive, irreversible impairment of the renal function. It is thus the most important feature of primary hyperparathyroidism, as it is potentially fatal.

The polyuria observed in primary hyperparathyroidism is due to the direct effect of hypercalcemia on the distal tubules. The water reabsorption is suppressed and the free water clearance increased. The polyuria is always associated with hyposthenuria demonstrable even in the early phase of the illness.

Hypocalcemia, hypophosphatemia and hypomagnesemia have been observed after removal of a functioning parathyroid adenoma with pronounced skeletal involvement. These features are caused by a rapid deposition of calcium phosphate in the skeleton. The so-called recalcification tetany is caused partly by the hypocalcemia, and partly by the hypomagnesemia.

The immunoreactive parathyroid hormone concentration (IPTH) is not always elevated in cases of primary hyperparathyroidism. Except

for the unique experience of REISS (1971), the literature reports a considerable overlap between normal and elevated levels (BERSON, 1966; ARNAUD, 1971). However, ARNAUD (1971) finds that when the serum calcium concentration is measured as well as IPTH, it is practically always possible to discriminate between normal and pathologic cases. In other words, when the serum calcium concentration is in the high normal range IPTH is low or undetectable in normal subjects, in contrast to the level in patients with primary hyperparathyroidism, where it is elevated. In secondary hyperparathyroidism of renal insufficiency IPTH can be in the same range as in primary hyperparathyroidism in some assays and much higher other assays. These differences are caused by the occurrence of at least two different molecular forms of parathyroid hormone in peripheral serum and site-specific antibodies. IPTH can be suppressed with calcium infusions in all cases of primary hyperparathyroidism, thus ruling out complete autonomy of parathyroid hormone secretion (POTTS, 1971). This is in contrast to the results of REISS (1971) and BUCKLE (1971), who seemed to be able to suppress IPTH in primary chief-cell hyperplasia of the parathyroid glands but not in parathyroid adenomas. In renal insufficiency IPTH is lowered following an increase of the calcium concentration in the dialysis bath (FOURNIER, 1971). IPTH has been suppressed with calcium in two cases of tertiary hyperparathyroidism (BRICKER, 1972; VOSIK, 1972). In conclusion, IPTH secretion can be suppressed with calcium in primary hyperparathyroidism, thus ruling out complete autonomy of PTH secretion. The pathogenesis of tertiary hyperparathyroidism (from secondary hyperparathyroidism) remains unknown.

5. Clinical Features and Symptoms

a) Presentation and Course

The *clinical picture* and the *course* of primary hyperparathyroidism are extremely variable. As well as the classic type with VON RECKLINGHAUSEN'S osteitis fibrosa, there is a form presenting with predominantly renal symptoms, and other forms with gastric ulcers, pancreatitis, gout, pseudogout, and band keratitis. There are other forms which show no signs of organic changes, but present clinically with functional symptoms only (hypercalcemic syndrome).

Finally, there is a symptomless form with hypercalcemia (chemical hyperparathyroidism). In addition, parathyroid adenomas discovered incidentally at thyroidectomy show all the morphological signs of a functioning adenoma without giving rise to clinical symptoms or hypercalcemia. The interpretation of these "silent" adenomas is still speculative, because of the lack of sufficient data (estimation of parathyroid hormone in the blood) (p. 907).

The course is chronic in most cases. Exceptionally it is short and peracute or there are acute phases during the chronic course (acute hyperparathyroidism). There have been reports of spontaneous necrosis of the parathyroid adenoma (p. 906). The frequency of these spontaneous healings is not known.

b) Skeletal Changes (Osteitis Fibrosa Cystica Generalisata von Recklinghausen)

The skeletal involvement was the predominant feature in the first recognized cases of primary hyperparathyroidism which were identified beyond doubt, but the skeletal changes are now considered quite an uncommon feature. Pronounced osseous changes can be seen clinically in about 15% of all cases of primary hyperparathyroidism (KEATING, HELLSTRÖM and RASMUSSEN, 1952). A considerably higher percentage is obtained, however, when more sensitive methods of skeletal examination are used. Skeletal involvement is found in 20–30% of cases by systemic X-ray examination (STEINBACH, 1961), and in 80% with the aid of bone biopsies after labeling with tetracycline (VAN DER SLUYSVEER, 1964). Quantitative microradiography of bone biopsies (JOWSEY) and biochemical methods with "bone-seeking" elements can detect involvement of the skeleton in all patients with primary hyperparathyroidism (radioactive calcium: BAUER, 1955; stable strontium: HARRISON, 1959).

Primary hyperparathyroidism occasionally presents clinically with bone symptoms only. They are, however, much more often associated with other symptoms of hyperparathyroidism, such as nephrolithisasis.

α) Clinical Symptoms of the Skeletal Involvement

The beginning of the skeletal disturbance is usually marked by the development of vague pains in the back, the sacral region, the hips and the limbs after prolonged exertion. Deformations results over some months, the most common being dorsal kyphosis, or kyphoscoliosis with the simultaneous formation of a pigeon chest. Shortening and widening of the neck so that the collar becomes too tight is very characteristic. Further characteristic changes are shortening of the trunk, with loss of the physiological lumbar lordosis, and the forma-

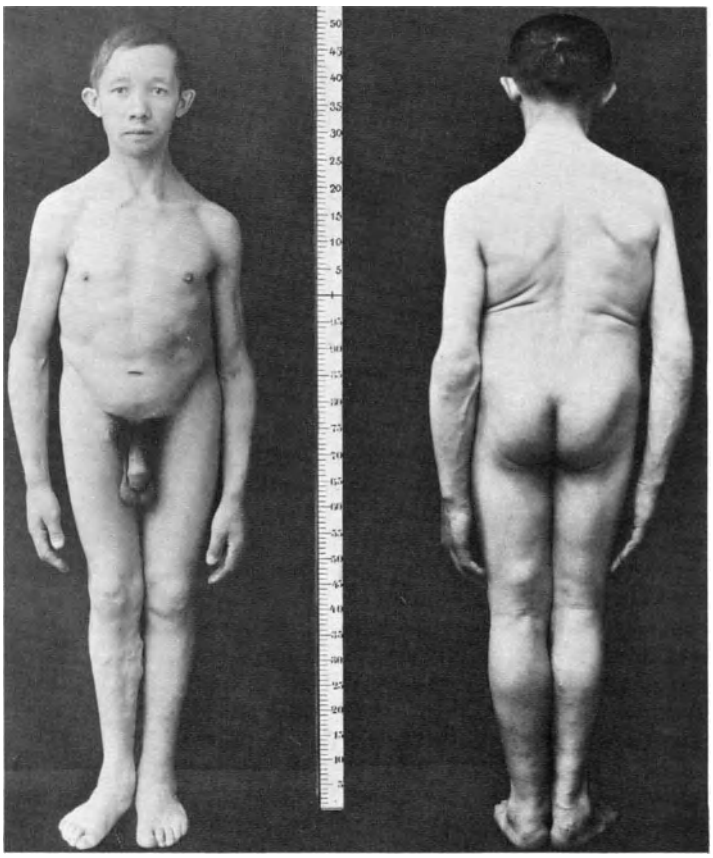


Fig. 25. Kyphoscoliosis, shortening of body and consecutive folding of the skin of the trunk in primary hyperparathyroidism. The hands reach down to the knees

tion of compression folds across the skin on the trunk. These patients appear to have arms which are much too long in relation to the trunk (Fig. 25). The ratio of half the arm span: the length of the upper part of the body: the length of the lower half of the body is no longer 1:1:1. Shortened, thickened fingers with an appearance reminiscent of clubbed fingers are also typical. In contrast to true clubbed fingers due to hypertrophy of the soft tissue, the cause in these cases is compression of the soft tissue due to acro-osteolysis (X-ray findings, p. 915). Slight thickening of the skeletal structures is also noted occasionally and is due to cysts in the bones and to osteoclastomas (X-rays, p. 915). An osteoclastoma can develop with no other demonstrable features as a circumscribed tumor of the jaw, in which case it is called an epulis. Not all epulides are due to hyperparathyroidism, but a thorough examination for hyperparathyroidism should be made nonetheless in every case with an epulis. Much too frequently an epulis is known about for years and is treated surgically as a giant cell tumor or by irridation. It may even be cured, while the fundamental disorder progresses further and in some cases develops into irreversible nephrocalcinosis (HELLSTRÖM and WELTI). In rare cases, the involvement of the jaw bones leads to sudden loosening of the teeth with subsequent loss of healthy teeth. The skeletal disturbance exceptionally presents first as a spontaneous fracture at the site of a bony cyst or of an osteoclastoma, or also away from such a focus. The tendency to heal, however, is good. When the fracture penetrates a thin-walled cyst, consolidation is sometimes possible only after the removal of the parathyroid adenoma (Fig. 29).

All these skeletal changes can be detected clinically, providing they are looked for. It is important to diagnose them, since they allow the exclusion of joint diseases, intervertebral disk disorders, and inflammatory and tumor-like disorders of the locomotor system. It must be remembered, however, that gout or pseudogout (chrondocalcinosis), i.e., a joint disease, is seen in about 5% of cases of primary hyperparathyroidism. The idea that a painful disorder of the locomotor system is due to either a skeletal

or a joint disease, but not to both at the same time, is not always correct.

The bone pains become very acute during the night in some advanced cases. In these cases they are not limited to the site of healed or badly consolidated fractures, but involve the whole skeleton. The stability of the skeleton can be reduced to the extent that the long bones and the pelvis become bent even in the absence of fractures. Nursing is thus made difficult, since every contact with the patient causes him intolerable pain. The patients usually die of the advanced nephrocalcinosis present at the same time.

β) X-Ray Examination of the Skeleton

This reveals primarily the generalization of the disorder (osteitis generalisata), and allows differentiation of circumscribed skeletal disorders (Sudeck syndrome, Jaffe-Lichtenstein disease, Paget's disease) from most metabolic disorders of the skeleton (osteoporosis, osteomalacia, primary hyperparathyroidism). In severe cases, the atrophy of the bone can be recognized at almost any part of the skeleton. The cortex appears to be rarified and porous. It may even be completely perforated and corroded. The spongiosa is not well defined. It looks like cotton wool and the radiological contrast is poor. X-ray of the lumbar vertebrae shows the typical biconcave fish vertebrae, and the bodies of the thoracic vertebrae are wedge-shaped and deformed. Fractures of the bones are common, and bending of the bones is less often observed. They can give rise to heart-shaped deformation of the pelvis.

In addition to the severe, generalized atrophy of the bones, a series of other symptoms are known to be specific to hyperparathyroidism: subperiosteal resorption, acro-osteolysis, bony cysts, osteoclastoma, and a ground-glass appearance of the skull. Occasionally they are also seen in a milder form in renal osteitis of secondary hyperparathyroidism (p. 936f.).

X-Ray Examination: Subperiosteal Resorption. This is an early symptom and one of great importance in the differential diagnosis from osteoporosis and osteomalacia in particular. In osteoporosis, even in the advanced stages, the cortex is rarified but still sharply defined. In osteomalacia, involvement of the cortex begins relatively late and is only slight (Fig. 26), while in hyperparathyroidism, subperiostal resorption zones and atrophy of the cortex can be ob-

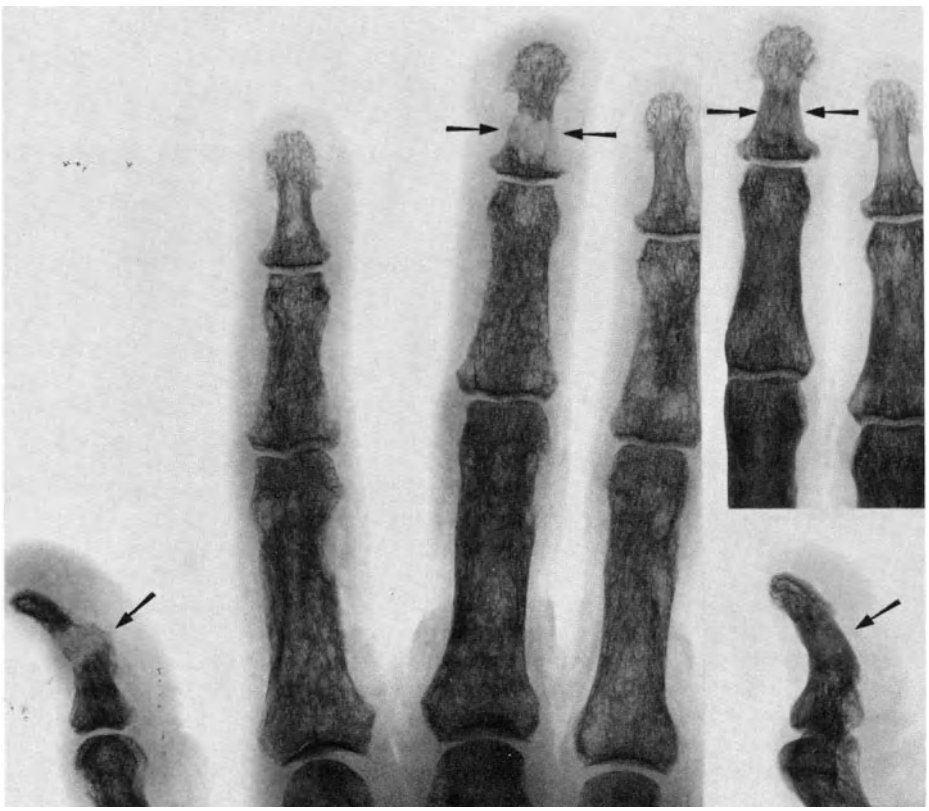


Fig. 26. General atrophy of bone and multiple zones of Looser in osteomalacia. Healing after treatment with Vitamin D.

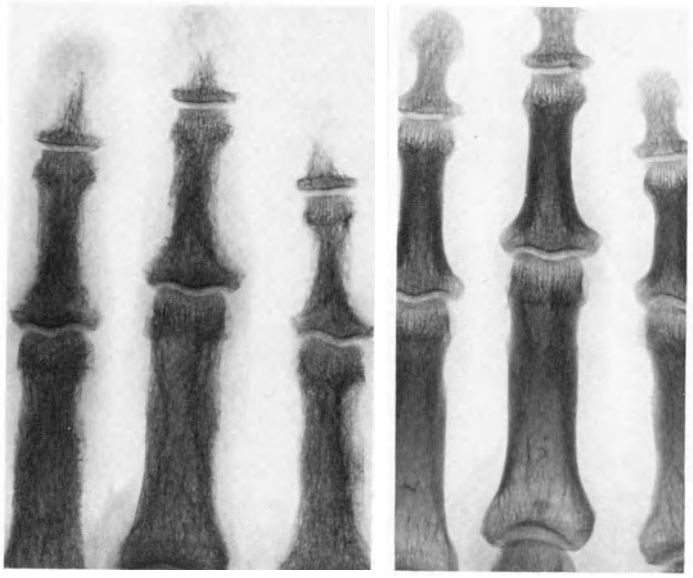
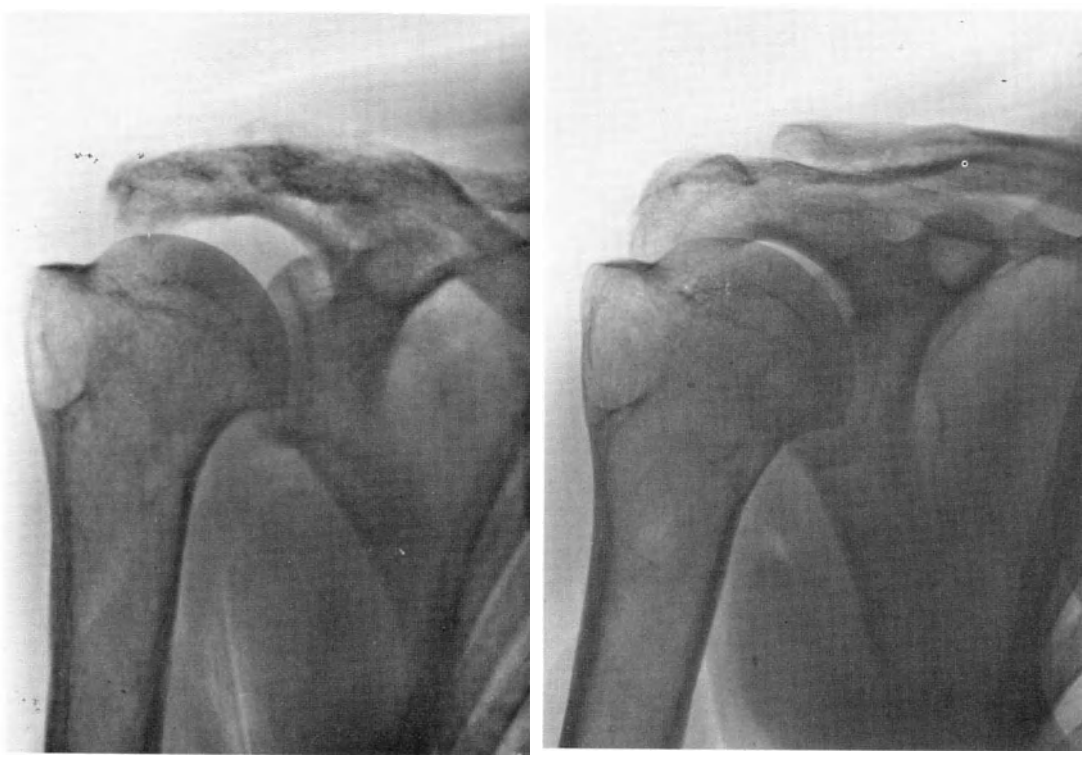


Fig. 27. Subperiosteal resorption and acro-osteolysis of the end phalangi of the fingers in primary hyperparathyroidism. Healing after extirpation of parathyroid adenoma

served at an early stage. The cortical atrophy is most impressive in the middle phalange of the middle finger (Fig. 27). The cortex appears to be very rarified or even finely granulated. The outer silhouette of the bone is no longer recognizable in many places, so that the spongiosa appears to form the edge. This phenomenon is referred to as spongio-



a b

Fig. 28. a) Acro-osteolysis of the acromioclavicular joint in primary hyperparathyroidism. b) Healing after extirpation of parathyroid adenoma

sation of the cortex. In other places, the cortex immediately below the periosteum is sometimes still present in parts but fully resorbed over other parts. These particularly typical, ribbon-like narrow defects sometimes extend for several millimeters parallel to the outer contour and are described as subperiosteal resorption (Fig. 27). Subperiosteal resorption can also be recognized in the cortex of the alveola of the teeth, where it is termed as loss of the lamina dura. Great stress used to be laid on this symptom, but it is unreliable since it is too dependent on the occurrence of paradentosis. A disappearance on porosity of the cortex can be seen in the bony walls of the paranasal sinuses (Fig. 31) and furthermore in the outer borders of the pubic and ischiopubic rami.

X-Ray Examination: Acro-Osteolysis. This term describes pronounced cortical resorption of multiple terminal bone structures. It is most severe in the distal phalanges of the fingers, where it can lead to the complete disintegration of the bony structures, so that only a few traces of the spongiosa scattered in the soft tissues are still recognizable (Fig. 5). The changes lead to clubbed fingers, with distension and deformation of the terminal phalanges, as has been mentioned already. Acro-osteolysis is occasionally also seen at the upper ends of the fibula and tibia, the pubic symphysis, at the sacroiliac joints and in a severe form in the acromioclavicular joints. The articular ends of the clavicle and the acromion appear disintegrated and corroded, their contours have a finely serrated appearance (Fig. 28). This feature is particularly valuable for diagnosis, since it is occasionally discovered at a routine chest X-ray in the absence of clinical symptoms or any suspicion.

X-Ray Investigations: Bone Cysts and Osteoclastomas (osteitis cystica). These are not mandatory findings, but their presence is an important diagnostic index of hyperparathyroidism. Differentiation between osteoclastomas and true cysts is not possible on X-ray plates, and they are often collectively termed as bone cysts. Bone cysts are sometimes found in the epiphyses of the long bones situated in the

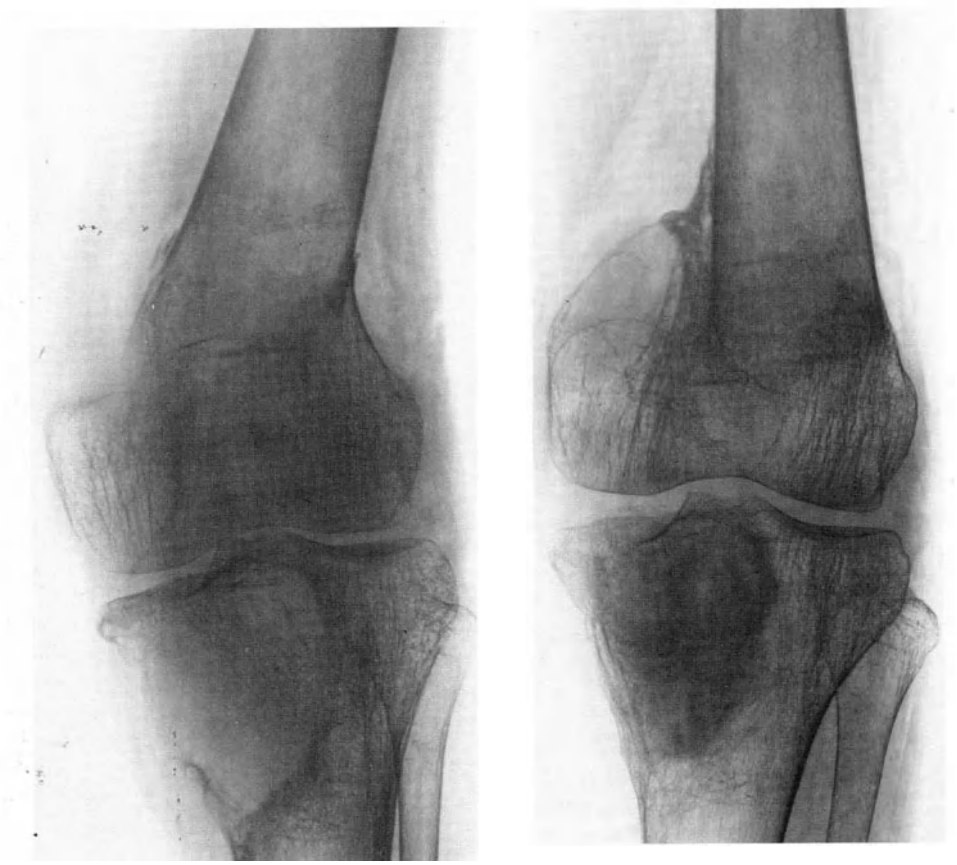


Fig. 29. Bone cyst and fracture due to cyst of the distal end of the femur; osteoclastoma of proximal end of tibia. Healing of the cyst, eburnization of osteoclastoma after extirpation of parathyroid adenoma

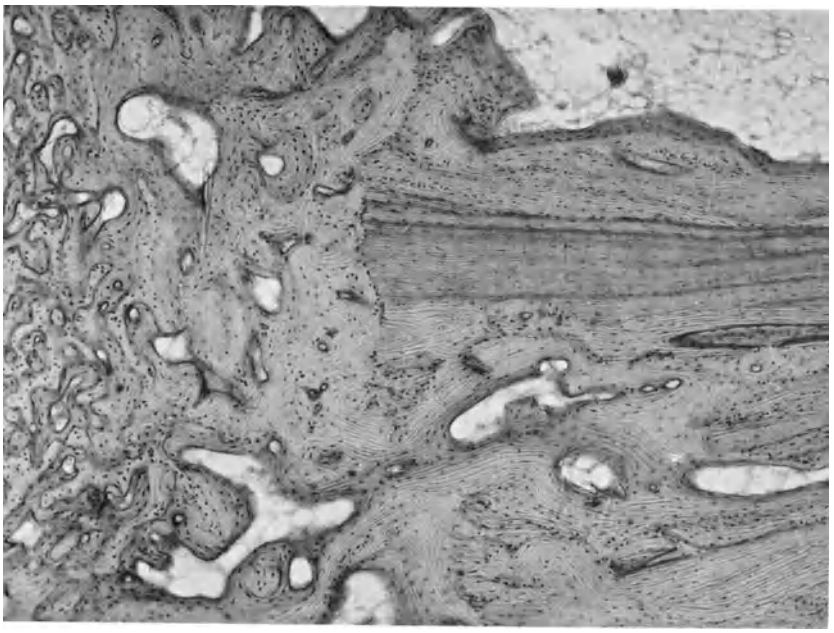


Fig. 30. Healing of a bone cyst in primary hyperparathyroidism after extirpation of parathyroid adenoma. Edge of cyst in middle of picture, right: old lamella, left: irregular zones of repair, new formation of bone

cortex or centrally. The most frequent localizations are the shoulder, the elbow, the distal end of the radius, the femoral head, the trochanter and the knee. Osteoclastomas are also found in the form of epulides in the jaw, in the cortex of the diaphyses of the long bones, in the ribs and in the phalanges. Traumatic cysts containing fluid due to hemorrhage of the marrow cannot be definitely differentiated from osteoclastomas by radiological examination. Both appear as sharply defined, round or polycyclic recesses in the structure of the bone, and can be uni- or multilocular. A tumor-like penetration is sometimes demonstrable in the cortex at the site of the bone cyst or of the osteoclastoma. During healing, which can occur spontaneously or after surgical removal of the functioning parathyroid adenoma, the osteoclastoma usually shows us an opaque eburnation (Fig. 29), whereas with true cysts only the margin of the cyst is seen at all clearly (Figs. 29, 30). Osteoclastomas and cysts can both be lost in the general faint pattern of the osseous tissue, and remain undetected during the active phases. After removal of the parathyroid adenoma, however, they are easily recognizable.

X-Ray Investigation: Ground-Glass Appearance of the Skull. The ground-glass appearance of the skull is due to the subperiosteal resorption as well as to multiple (microscopically) small osteoclastomas. Radiological examination shows a scattering of fine granules, and in advanced cases a coarse perforation of the spongiosa, giving a moth-eaten appearance. The internal and external cortex are almost completely lost (Fig. 31). Not uncommonly, the skull appears to be thickened, giving the impression of osteosclerosis. This process of sclerosis of the cranium is found predominantly during healing after removal of the parathyroid adenoma, but has also been observed in chronic advanced cases of hyperparathyroidism. The picture of cranial sclerosis in the active and healing stages has often been mistaken for Paget's disease. Differentiation is possible, however, if it is borne in mind that foci of patchy thickening are almost always observed as well as resorption in Paget's disease, in contrast to hyperparathyroidism (LIEVRE, 1959).

X-Ray Investigation: Osteosclerosis. Osteosclerosis occurs, as mentioned above, in the skull, and also sometimes in larger skeletal areas such as the pelvis (AITKEN). It can be difficult to differentiate from osteosclerosis of Paget's disease (PLATTNER). Generalized osteosclerosis, or so-called banded sclerosis of the lumbar vertebral bodies, is more often seen in secondary than in primary hyperparathyroidism (p. 937).

γ) Histology of the Skeletal Changes (Osteitis Fibrosa, Osteomalacia)

In primary hyperparathyroidism affecting the skeleton, the most important morphological

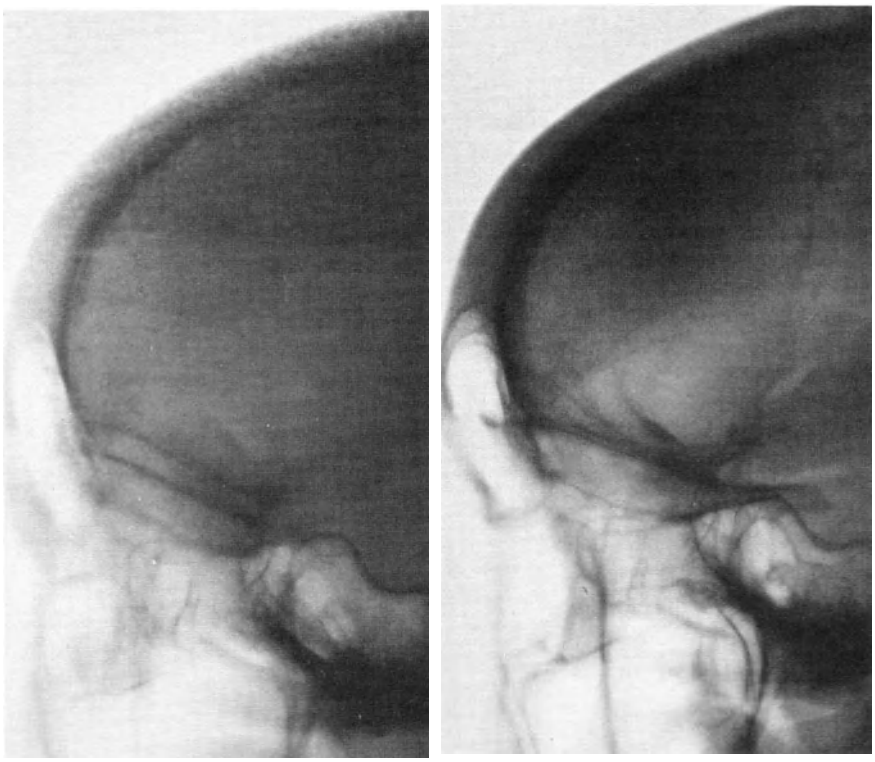


Fig. 31. Ground-glass appearance of skull. Rarification of corticalis of septs of sinuses in primary hyperparathyroidism. Healing after extirpation of parathyroid adenoma

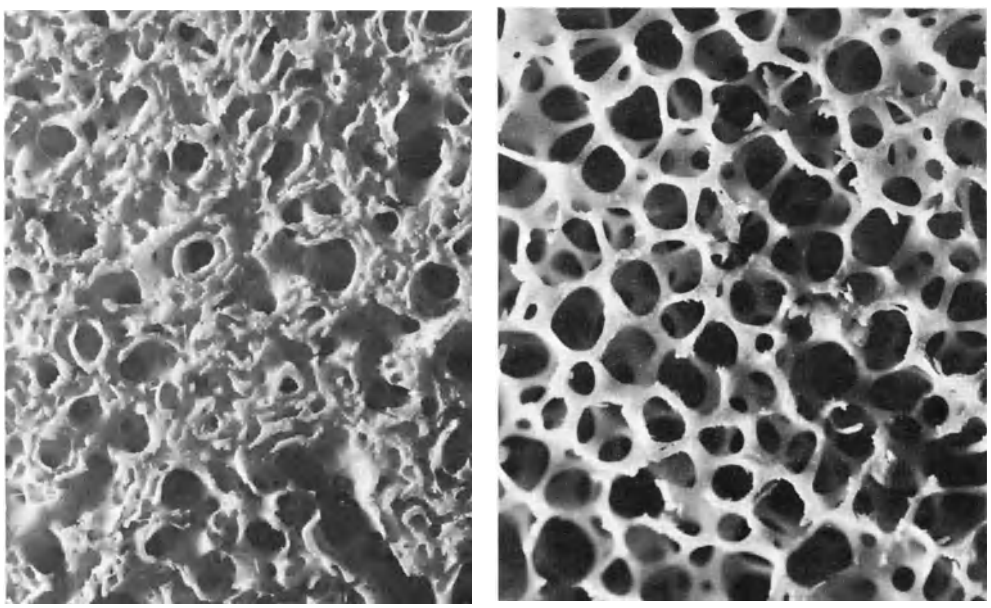


Fig. 32. Left: dissecting resorption in primary hyperparathyroidism. Magnified picture of cross-section of vertebra (maceration preparation). Right: normal vertebra

feature is osteitis fibrosa. A gross increase in osteoid tissue (osteomalacia) is only rarely observed.

Osteitis fibrosa begins in the endosteum, surrounding all the trabeculae of the spongiosa, and filling the Havers' canals in the cortex. The endosteum grows steadily from a layer which is hardly visible microscopically into a thick broad band of connective tissue. A large number of osteoblasts and osteoclasts are found

on the surface of the bone. In the advanced stages, all the osseous trabeculae are covered by a thick layer of connective tissue. This fibrous tissue then penetrates right into the center of the osseous trabeculae by fine recesses of resorption. Osseous trabeculae become excavated to form tunnels over long stretches. This process of tunnel formation is called dissecting resorption (Fig. 32). In some places, the skeletal tissue becomes completely dissolved; irregular fields of connective tissue in which numerous osteoclasts are scattered are then found. When the fields with no skeletal substance are very extensive, osteoclastomas ranging from microscopic size to the size of an apple develop. The space of the hematopoietic marrow is encroached by endosteal fibrosis, so that anemia can develop.

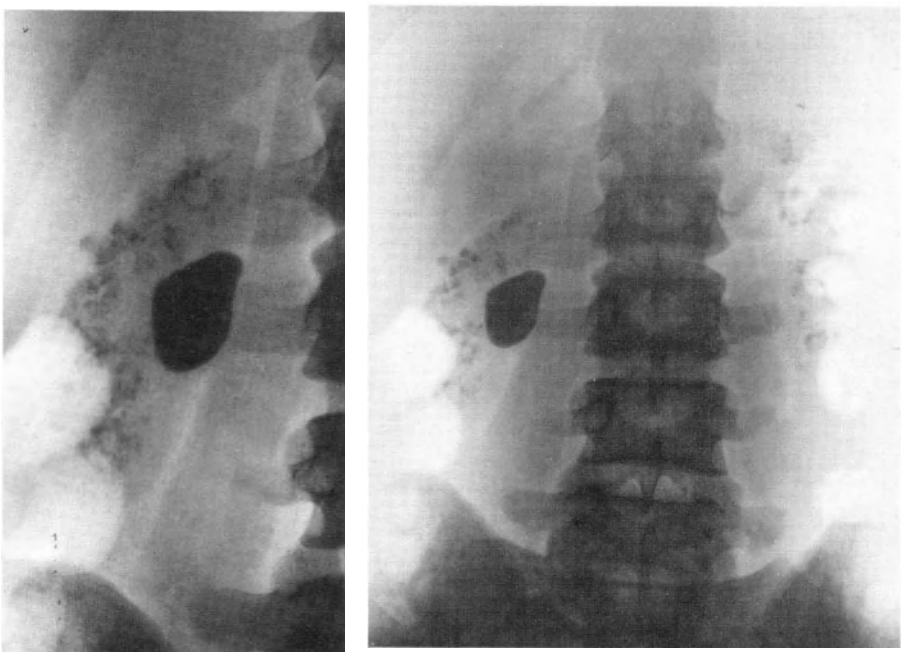
The osteomalacic component of the osteopathy is less often observed. Slightly widened osteoid margins are often seen corresponding to the greatly accelerated bone metabolism. Severely widened osteoid margins, indicating clinical osteomalacia, are only rarely observed (LICHTWITZ, 1957). They are always found in association with osteitis fibrosa as a mixed osteopathy.

There is still little known about the pathogenesis of the fibro-osteoclastic, osteomalacic and mixed osteopathies of primary hyperparathyroidism. It involves the interaction of numerous factors, which is partly synergistic and partly antagonistic. These factors are 1. the severity and the duration of the parathyroid hyperactivity, 2. the exogenous intake of calcium, 3. the intestinal absorption of calcium, 4. the impairment of the glomerular filtration of calcium or of the tubular reabsorption of calcium due to the hypercalcemia (Chap. XIV, p. 852, p. 910), 5. frequently renal insufficiency, resulting in a fall of the serum calcium, and 6. possible counterregulation by calcitonin.

c) Nephrolithiasis

Nephrolithiasis is by far the most frequent clinical symptom in primary hyperparathyroidism, both in the earliest stages and in the later course. It is found in about 75% of cases of hyperparathyroidism. The figures reported vary widely according to the classification of the patients (medical, urological, psychiatric, outpatients or inpatients). KEATING gives a frequency of 76.8% in the 380 cases seen at the Mayo Clinic. Solitary renal stones were found in 14.7%, multiple renal calculi in one kidney in 28.2%, and bilateral renal stones in 33.9%. In many cases nephrolithiasis is the only symptom of hyperparathyroidism. In addition, nephrolithiasis can occur in combination with any other signs of the hypercalcemic syndrome and with skeletal involvement.

The proportion of cases of primary hyperparathyroidism among unselected cases of



a b

Fig. 33a and b. Nephrocalcinosis and nephrolithiasis in primary hyperparathyroidism

nephrolithiasis is also the subject of speculation. Figures given vary a great deal. Most authors accept 5% as the minimum, and even 12% in cases of recurrent nephrolithiasis. McGeown in Belfast has indicated 18%, but this has not been confirmed in other regions.

Any attack of renal colic can be an early symptom of primary hyperparathyroidism. The second attack of renal colic or additional clinical symptoms of the disease may not occur for some years. However, renal colic sometimes recurs at short intervals. The renal calculi formed in primary hyperparathyroidism consist of calcium oxalate or calcium phosphate. Concretions of ammonium and magnesium phosphate can be observed in the presence of an intercurrent urinary infection. This is rare in the early stages of the illness. Urate stones are found at the same time as or alternating with stones containing calcium (Howard, 1954). The renal calculi range in size from simple gravel in the urine to solitary stones. They may also form casts, or multiple caliciform stones.

The outcome of nephrolithiasis varies widely from one individual to the other. Patients with history going back over decades sometimes show no signs of renal damage at the time of the parathyroid operation. In other cases, the condition progresses to bilateral infection of the kidneys with pyonephrosis. The prognosis of the nephrolithiasis is in general good after a parathyroid adenoma has been successfully removed. Regression of the calculi is possible after the hypercalciuria has been corrected. In many cases, numerous renal stones are excreted over the years, and any concretion remaining in the renal pelvis usually remains silent even in the presence of urinary tract infection. Recurrences or the further growth of existing stones have rarely been observed. Lithotomy should be performed only after parathyroid surgery.

d) Nephrocalcinosis

Nephrocalcinosis is much less common than nephrolithiasis (7.1%, Mayo Clinic), but the prognosis is much more serious. It has been stated that nephrocalcinosis is observed only rarely in association with nephrolithiasis. This, however, is not an absolute rule (p. 918, Fig. 33). The clinical diagnosis is made on radiological examination, which shows a few scattered or numerous dots of calcium lying very close together in the renal shadow, predominantly in the renal pyramids (Fig. 33). The comparison of X-rays made during life with those of the isolated organ at autopsy shows, however, that the nephrocalcinosis is already advanced before it can be detected on a straight abdominal X-ray or a tomogram. The early stage of the condition is not even indicated by the histological demonstration of calcification, since in experimental nephrocalcinosis the histological deposition of calcium is preceded by a very definite increase in the content of the chemically measurable calcium in the kidney. It must therefore be assumed that the first stage in the renal disorder is represented by a submicroscopical change in the tubular epithelium and in the basal membrane of the tubule. All the other stages up to complete calcification of all the epithelial tubules, the basal membranes and large areas of interstitial tissue occur after this (Engfeldt). Radiological evidence always means that the condition is at an advanced stage. We have often observed that polyuria associated with hyposthenuria is usually present at an early stage in primary hyperparathyroidism (p. 910). This loss in concentrating capacity sometimes disappears completely after the parathyroid adenoma has been removed, but sometimes it continues, especially if nephrocalcinosis is present, so that the cure obtained in primary hyperparathyroidism is only partial or temporary in some cases. It can progress to tubular disturbances and to severe glomerular insufficiency with uremia and malignant hypertension. The morphological changes causing late renal damage can be obstruction of large tubular areas and compression of the medium-sized arteries by the calcium deposition. Death follows from uremia or from a vascular complication of the hypertension. Too many cases of hyperparathyroidism are diagnosed too late and lead to fatal uremia or malignant hypertension, in spite of a successful parathyroidectomy. Only early diagnosis can avoid this.

e) Gastric Ulcer

A peptic ulcer of the duodenum or of the stomach is found in about 15% of cases of primary hyperparathyroidism. Hyperparathyroidism is therefore associated with an increased predisposition to the development of ulcers. The course of gastric ulcers occurring in hyperparathyroidism appears to be more severe than that of gastric ulcers occurring otherwise. As a rule, the clinical and radiological findings disappear rapidly after the removal of a parathyroid adenoma. It appears that the increase of the gastric secretion is due to the hypercalcemia (Barreras). The association of an ulcer with a parathyroid adenoma is of practical importance, and the possibility of primary hyperparathyroidism must always be considered in every case of peptic

ulcer which is difficult to treat (FRAME). In exceptional cases hyperparathyroidism may even be discovered because of a peptic ulcer. Treatment of an ulcer with milk and alkaline salts is not only useless but also dangerous in the presence of primary hyperparathyroidism as it can lead to hyperparathyroid crisis by the summation of two hypercalcemic factors: hyperparathyroidism + milk-alkali syndrome. Primary hyperparathyroidism must always be considered when short-term treatment of an ulcer with milk and alkaline salts causes an unfavorable reaction with nausea and vomiting.

Peptic ulcers in the Zollinger-Ellison syndrome are discussed in Chap. XV (p. 971).

f) Pancreatitis

Pancreatitis is observed more frequently in primary hyperparathyroidism than among healthy individuals chosen at random. KEATING found it 10 times among 380 cases at the Mayo Clinic (2.6%). More recent investigators give higher figures (MIXTER 7%, KYLE 12%, LUDWIG 19%), which may possibly be due to the fact that even patients with mild forms of pancreatitis are very carefully examined for hyperparathyroidism. All forms of pancreatis can be present in primary hyperparathyroidism: acute recurrent, subacute recurrent, chronic with abdominal pains, or chronic without pain, but with nausea, vomiting, diabetes and steatorrhea. During the course of acute pancreatitis, hypocalcemia and hypophosphatemia develop almost regularly during the first or second day, reaching the lowest levels between the fourth and seventh days. So far, hypocalcemia has been attributed to the binding of large amounts of calcium in the region of the necrotic adipose tissue, and also to activation of trypsinogen due to the elevated serum calcium ions concentration. Recently, however, PALOYAN has put forward the view that the massive release of glucagon demonstrated during attacks of pancreatitis is responsible for the hypocalcemia (the action of glucagon causing a fall in the serum calcium and a release of calcitonin). It is important to diagnose hypocalcemia after pancreatitis, since it may mask the hypercalcemia of primary hyperparathyroidism. Primary hyperparathyroidism must be considered in every case of pancreatitis, and the serum calcium should not only be estimated during the acute phase but several times 1–3 months afterwards.

PALAYON considers the hypocalcemia which has been postulated but never confirmed in chronic pancreatitis is a cause of secondary hyperparathyroidism.

g) Band Keratitis, Conjunctival Calcification

Superficial deposition of calcium in the Bowman's capsule of the cornea has been observed in all forms of hypercalcemia, including primary hyperparathyroidism. As a rule, the calcifications are seen only in the lateral region of the palpebral fissure, in the form of ill-defined, patchy grey opacities, usually in a sickle-shaped formation along the margin of the cornea. This condition can be differentiated from arcus senilis by the very superficial localization of the lesions. The initial stages can only be recognized with the aid of a slit-lamp. Occasionally, subepithelial, superficially situated, round glass-like particles consisting of calcium are also found in the conjunctiva in the region of the palpebral fissure. The conjunctiva is nearly always inflamed in the region of the particles, and the patient complains of conjunctival irritation.

Very intensive marginal keratitis, with identical morphology, involving the entire palpebral fissure like opalescent glass, is occasionally found in the absence of hypercalcemia. It occurs as a dystrophic calcification in intraocular inflammation of different types.

h) Hyperuricemia and Gout

Hyperuricemia and gout are more frequently observed in patients with primary hyperparathyroidism than in the average population (hyperuricemia in approximately 50–60% of all cases of hyperparathyroidism, gout in 5–25% depending on the author). However, it is still not known whether the hyperuricemia and the gout are results of the renal insufficiency in primary hyperparathyroidism (SCOTT), or whether there is a genetic relation between gout and hyperparathyroidism (MINTZ, TURRIAN). It is also possible that the transport of uric acid through the renal tubules is impaired by the calcification, as is supported by the fact that cases of gout have also been described in hypercalcemia of sarcoidosis, and in pseudohypoparathyroidism with calcification of the soft tissues (SCOTT, 1964). In the differential diagnosis, it must be remembered that the articular changes cannot be interpreted as an argument against primary hyperparathyroidism, since attacks of gout and chronic gout of the joints are to be expected in a certain number of cases of hyperparathyroidism. Systematic investigation of every case of hyperuricemia and of gout for primary hyperparathyroidism has so far proved disappointing, however: SCOTT (1964) for example, did not detect a single of hyperparathyroidism in 100 cases of gout.

i) Pseudogout (Articular Chondrocalcinosis)

Like gout, pseudogout is found more frequently in patients with primary hyperparathyroidism than in the average population (in 2.5–7.5% of all cases of hyperparathyroidism, McCarty). The acute form of this clinically uncommon condition shows great similarity with an attack of monarticular gout, but it is the knee joint and not the big toe which is most often involved, and colchicin has no therapeutic value. In addition to the acute form, there is a chronic, polyarticular course. The fluid obtained from the joint during the acute phase contains numerous small crystals of calcium pyrophosphate, which almost proves the diagnosis (Mc Carty). The same crystals can also be demonstrated scattered diffusely or localized in articular cartilages (Aitken), ligaments, tendons and menisci. On radiological examination, they appear as a fine punctate haze, as bands, or as lines. Pseudogout does not only occur frequently in primary hyperparathyroidism, but also in diabetes mellitus (40–60%), whereas classic gout occurs in 4% and hyperuricemia in 35% of cased. It an be concluded that the deposition of calcium crystals is only partly due to the hypercalcemia of primary hyperparathyroidism (McCarty).

j) The Hypercalcemic Syndrome

This term includes a series of clinical symptoms arising directly from hypercalcemia, which are not associated with marked morphological changes, and usually disappear rapidly after correction of the hypercalcemia. The hypercalcemic syndrome does not only develop in primary hyperparathyroidism, but has also been observed in hypercalcemia of different etiologies. The clinical symptoms are: mental and neurologic changes, polydipsia, polyuria, loss of appetite, meteorism, vomiting, constipation, muscular weakness, and bradycardia. According to general opinion, skeletal and renal changes, peptic ulcers, gout, pseudogout and pancreatitis are not part of the hypercalcemic syndrome. However, symptoms of hypercalcemic syndrome usually are associated with the classic symptoms of primary hyperparathyroidism, i.e. the skeletal and renal changes, but they can also occur on their own, and according to Keyne's estimate, do so in 15% of all cases. Mild hypercalcemic symptoms are not noticed by many hyperparathyroid patients. Only after successful surgical treatment with the re-establishment of normal conditions, do they notice in retrospect that numerous symptoms have disappeared.

α) The Hypercalcemic Syndrome: Psychic and Neurologic Changes

The accounts in the medical literature are limited to case histories or enumeration of single symptoms such as depression, apathy, agitation, hallucinatory phenomena, and paranoia.

Bleuler was the first to attempt to classify the various mental changes in endocrine diseases. He found initially that mental changes could arise with almost all endocrine disorders, and also showed that these mental changes were on the whole similar to each other, regardless of the underlying endocrine pathology. He divided them into two well-defined forms according to their psychopathologic picture. The first form is a chronic type, the so-called localized psychosyndrome in the brain. Bleuler called it an *endocrine psychosyndrome*. There is considerable evidence that the mental changes observed in this condition are due to localized metabolic disturbances in the brain (similar psychic pictures are seen after psychosurgery, after localized cerebral inflammation, in inherited systemic degeneration, and in experimental investigations on the localized effects of hormones on different parts of the brain). The clinical picture takes the form of changes in mood, and changes in initiative and the individual drives. The second form of the endocrine psychic disorder is observed much less frequently and is due to diffuse brain damage. Its psychopathology is of the *acute, exogenous reaction type* (Bonhöffer). It presents clinically as a state of confusion or somnolence progressing into pre-coma or coma, or as a state of excitement or confusion with exaggerated emotions, hallucinations, dreams and delusions.

In primary hyperparathyroidism, it is usually the changes associated with the endocrine psychosyndrome which are observed. Its incidence is difficult to estimate. Petersen, a student of Bleuler, made a thorough investigation of 54 cases, and found significant disturbances in one third, mild psychic peculiarities still within normal limits in another third, and no deviations from normal in the last third of his hyperparathyroid patients. In general, severe changes are predominantly observed in the presence of highly elevated serum calcium concentrations (Petersen). The patients do not really appear to be ill, but seem affected, depressed, peculiar or childish. The changes in their mood and drive can be chronic, but they characteristically arise without warning, and disappear after some time just as unexpectedly. The patients are occasionally euphoric, irritable and spiteful, but more often they are depressive. The patient feels he is not fit to live, loses all

motivation, gives up fighting for his existence and say he wishes to die. From here onwards, all intermediate stages up to severe depression are found. The patients often also complain of mild symptoms such as tiredness, headaches, and confusion. In other cases, exaggerated sensitivity and irritability are the predominant features. These deviations usually all disappear a few weeks to 6 months after removal of a parathyroid adenoma. Changes in individual drives, such as hunger, thirst and aggressivity, are relatively frequent in primary hyperparathyroidism. It must be assumed that the polyuria and the polydipsia are also due to a renal mechanism (p. 910). Loss of appetite can be considered a disturbance in the drive, and also a result of the meteorism and constipation, which are common in primary hyperparathyroidism. The disturbance of the emotional state is thus much more pronounced than the changes in the individual drives in the endocrine psychosyndrome.

KIND, an associate of BLEULER, has already described several typical cases of the *acute, exogenous reaction type* (BONHÖFFER), which generally occurs only if the serum calcium concentration is over 16 mg/100 ml, in primary hyperparathyroidism. The main symptoms are memory disturbances, clouding of consciousness, drowsiness, disorientation and confusion. Most reports in the medical literature relate the organic confusion and disorientation to paranoia and schizophrenia. This is incorrect. These psychic changes, although due in general to severe, diffuse brain damage, can also disappear rapidly after treatment of the hypercalcemia.

Finally, some patients suffering from hyperparathyroidism with slight psychic disorders go through a hypocalcemic phase after removal of the parathyroid adenoma, and can be precipitated into a severe state of confusion. The reactions of these patients to treatment are quite variable. The emotional disturbance can disappear instantaneously after the serum calcium has been normalized. In some cases, the disappearence is only temporary, and the condition recurs after a short time. The further course of the condition is unpredictable. The state of confusion varies without any clear connection with the treatment carried out, and even without any correlation with the level of the serum calcium. Recovery may occur suddenly for no obvious reason (KIND and WERNLY, 1942). According to BLEULER, either hyperparathyroidism and hypoparathyroidism lead to similar psychic symptoms, or the psychic changes are related to the magnesium and not to the calcium levels, as hypomagnesemia also occurs in the postoperative state.

In addition to the mental changes, neurological disturbances have been observed in primary hyperparathyroidism, predominantly headaches and muscular weakness accompanied by sensory and motor disturbances of the peripheral nerves. The headaches occur frequently and are not always related to intercurrent hypertension. They do not respond to conventional analgesics but usually disappear quickly after removal of a parathyroid adenoma. The muscular weakness is restricted to generalized flaccidity, asthenia and increased fatigability on physical exertion, but exceptionally it becomes very severe, so that use of the extremities and in particular the ability to walk may be greatly impaired. The tendon reflexes are normal or slightly diminished, and diffuse muscular atrophy is occasionally found. GERSTER (1969/70) describes neuralgic pains, disturbances of the deep sensibility, of the conductance of peripheral nerves, and of the electromyogram. All these features, which are characterized mainly by severe hypercalcemia, can be cured if the parathyroid adenoma is removed within some few days or weeks, and the disturbances can disappear completely.

The increase of the protein content of the cerebrospinal fluid is of interest. It occurs in hypercalcemia of various etiologies, including primary hyperparathyroidism. In addition to primary hyperparathyroidism, the following pathologic conditions have been given as causes: idiopathic hypercalcemia, carcinoma without metastases in the brain, vitamin-D intoxication, milk-alkali syndrome (EDWARDS, 1959; KRAWITT, 1965). Only a small number of observations have been recorded, and changes in the CSF have not been seen in every case of hypercalcemia.

β) Polyuria, Polydipsia

Mild polyuria and polydipsia are frequently seen in primary hyperparathyroidism, but are rarely so severe that the patient complains of pronounced thirst. If the patient does experience thirst it is more tormenting than most other forms of thirst. It continues day and night. The patient declines milk, tea and lemonade, and requests cold water. However, no amount of fluid quenches the thirst and the feeling of discomfort remains. In contrast to this condition, the thirst encountered in diabetes insipidus is reduced by an adequate fluid intake. The polyuria in the hypercalcemic syndrome can reach 10 liters, and can be confused with diabetes insipidus. The polyuria, however, is resistant to vasopressin, and is sometimes associated with a loss of the sweat secretion.

Removal of the parathyroid adenoma reestablishes normal sweating.

The etiology of the polyuria and polydipsia has been dealt with on p. 876 and p. 910. The reader is referred to p. 932 for the effect produced on them by removal of a parathyroid adenoma.

γ) Anorexia, Flatulence, Vomiting, Constipation

Discrete forms of meteroism and constipation are almost always present in primary hyperparathyroidism. The patients often mention loss of appetite and nausea. There is a history of periodic attacks of vomiting, occurring relatively often for no apparent reason. The patients also complain of abdominal pains, even in the absence of an ulcer (p. 919) or pancreatitis (p. 920). The gastrointestinal symptoms are related to the reduction in neuromuscular excitability and are thus a direct result of the hypercalcemia. However, it has not been confirmed that cases with severe hypercalcemia suffer from intestinal disorders more frequently than those with less severe hypercalcemia. Nevertheless, nausea, loss of appetite, vomiting and loss of weight are never absent in severe hypercalcemia. Severe vomiting always implies the onset of the very dangerous acute form of hyperparathyroidism (p. 923 f.).

δ) Circulatory Symptoms

Bradycardia is observed most frequently and must be mentioned first, followed by the electrocardiographic findings. The typical ECG findings for hypercalcemia, with a shortening of the Q-T interval, are less often encountered than the prolongation of the Q-T found in hypocalcemia. The hypercalcemia can therefore rarely be diagnosed from the ECG alone. The fact that an increase in the calcium ion concentration in the extracellular space enhances the effect of digitalis is of practical importance. Great caution must therefore be exercised in using digitalis and strophantine in hyperparathyroid patients with hypercalcemia. The same is true of adrenaline.

k) Acute Hyperparathyroidism

A very dangerous, acute exacerbation of hyperparathyroidism may suddenly arise without apparent cause. This acute phase is similar to the state caused by experimental poisoning with parathyroid hormone, and is known as acute hyperparathyroidism or parathyroid crisis. An excessive concentration of calcium in the blood is the initial characteristic biochemical finding. In the later course of the crisis, severe hyperphosphatemia also develops. The parathyroid crisis can develop from the chronic cases of previously existing primary hyperparathyroidism slowly or unexpectedly. Sometimes, however, it occurs suddenly without any premonitory symptoms. The situation usually becomes serious when the calcium level exceeds 17 mg/100 ml. The highest recorded values are about 21 mg/100 ml. Shortly before the crisis, there is vomiting or polyuria, or both. In the acute condition, there is severe generalized dehydration. Circulatory collapse, renal failure, hyperpyrexia, confusion, hallucinations, somnolence and coma have been observed. Hyperphosphatemia develops as a result of the renal failure. The simultaneous oversaturation of the serum with calcium and phosphate in a state of severe dehydration leads to the deposition of calcium in almost all the tissues, kidneys, lungs, gastric mucosa, arteries, periarticular tissues, conjunctiva, cornea, tympanic membrane, myocardium and skeletal muscles, parotids, pancreas, thyroid and liver. Thromboses are occasionally found, most often in the arcuate and interlobular veins of the kidneys; according to STEMPL, this is another factor in the etiology of the terminal uremia. Hemolysis and parenchymatous jaundice have been reported, as has intravascular clotting.

It has been postulated that an acute rise in the release of parathyroid hormone is the cause of the acute crisis. In this light, the clinical picture is identical to that of experimental parathyroid hormone poisoning. It has also been repeatedly observed that crude palpation of a parathyroid adenoma can precipitate a parathyroid crisis. In recent years, however, it has become apparent that the excess parathyroid hormone is not the only factor and probably not even the most important cause of a parathyroid crisis. More frequently acute hypercalcemia arises in the presence of known or unknown primary hypercalcemia when a second pathogenic mechanism favoring the development of hypercalcemia is introduced, such as the administration of vitamin D. Parathyroid hormone and vitamin D act synergistically to produce mobilization of calcium from the bones, and the calcium mobilization is potentiated by the presence of elevated concentrations of both parathyroid hormone and vitamin D. A similar potentiation can be caused by the following pathogenic factors: postoperative immobilization, BLACK warns that surgery should never be performed in patients with primary hyperparathyroidism unless the patients are well hydrated and the serum calcium lowered if possible. Hyperparathyroidism can be potentiated by high doses of oral calcium (COLLIP),

by chlorothiazides and treatment with milk and alkaline. The unfavorable effect observed in the treatment of peptic ulcers in primary hyperparathyroidism and all other factors which can be considered as possible causes of hypercalcemia are summarized in Section α, p. 927.

Treatment of the parathyroid crisis has been dealt with on p. 933.

6. Diagnosis

The diagnosis of primary hyperparathyroidism is based largely on laboratory findings, but clinical diagnosis is sometimes possible: the clinical suspicion gives the first impetus, the laboratory investigations confirm or contradict the clinical suspicion. There are no serious difficulties in recognizing the advanced forms of the illness with extensive skeletal and renal involvement. Care and thoroughness are necessary for early diagnosis of the disease, which is only possible if the doctor considers the possibility of primary hyperparathyroidism when faced with a series of apparently trivial symptoms such as renal colic, spontaneous fractures, vague abdominal symptoms, gastric ulcer, and tiredness, headaches and depression. Special attention should be given to patients who present with several suggestive symptoms. Table 8 gives a survey of the clinical, radiological and laboratory features considered as positive evidence.

Finally, there are cases of clinically symptomless hyperparathyroidism, which can only be detected by systematic estimation of the serum calcium and parathyroid hormone concentrations. The conclusive investigations carried out by BOONSTRA have already been mentioned above (p. 904). It is thus advisable to estimate the serum calcium at least once in every patient admitted to hospital.

Laboratory Tests. Repeated demonstration of hypercalcemia is the most important step in the diagnosis of primary hyperparathyroidism. The existence of a normocalcemic form of primary hyperparathyroidism with recurrent nephrolithiasis as an isolated clinical symptom is discussed in the section on the differential diagnosis of idiopathic hypercalciuria on p. 929. The detection of hypophosphatemia or hypercalciuria is considerably less reliable. Up to now, the only single test of parathyroid function which also allows a conclusive diagnosis is radioimmunologic determination of the parathyroid hormone concentration in the serum. A critical account of these tests is given on p. 941. The tests measuring the phosphate clearance must be used cautiously. In many cases, the diagnosis of hyperparathyroidism is made by a process of elimination when no other cause for hypercalcemia can be found. The cortisone test after DENT is the best method for differentiating between hypercalcemia due to hyperparathyroidism and that due to some other factor, but this method is not infallible.

Table 5

Clinical evidence
Renal colic, concretions containing calcium
Polyuria, polydipsia
Sacral and limb pains
Thoracic kyphosis, compression folds on the trunk
Spontaneous fractures
Clubbed fingers
Tumors of mandible and maxilla (epulis)
Anorexia, meteorism, intermittent abdominal pains or dyspepsia
Peptic ulcers refractory to therapy
Vomiting after milk-alkali treatment
Recurrent pancreatitis
Asthenia, muscular adynamia
Tiredness, malaise, headaches
Depression, irritability, aggressiveness
Confusion, hallucinations
Somnolence, apathy, coma with hyperpyrexia
Band keratitis, subconjunctival calcifications
Gout, hyperuricemia, pseudogout
Tetany in newborns (maternal hyperparathyroidism)
Radiological evidence
Generalized skeletal demineralization
So-called osteoporosis
Spongiosation of the cortex
Subperiosteal resorption of the phalanges
Acro-osteolysis (fingers, acromioclavicular joints)
Bone cysts
Epulis
Ground-glass appearance of the skull
Nephrolithiasis
Nephrocalcinosis
Laboratory evidence
Elevated level of immunoreactive parathyroid hormone in the serum
Hypercalcemia
Hypophosphatemia
Elevation of the alkaline phosphatase
Hypercalciuria (24-hour urine)
Unexplained uremia
Unexplained isosthenuria
Shortening of the Q-T interval in the ECG

The localization of a parathyroid adenoma can rarely be determined by palpation, and a thyroid node cannot be excluded. Most intrathyroidal parathyroid adenomas are situated in the lower pole of the thyroid, which can be significantly enlarged on palpation. The radiological identification of a parathyroid adenoma is rarely sucessful, mostly in cases of large cystic adenomas in the mediastinum.

Arteriographic and scintigraphic methods are equally unsuccessful so far. Better results were obtained, however, by preoperative cathe-

terization and radioimmunological measurement of the parathyroid hormone concentrations in 8 large veins of the neck and of the superior aperture of the thorax (REITZ, 1969). REISS and CANTERBURY (1969) used the immunoassay for parathyroid hormone, introducing a different method for the lateral localization of parathyroid adenomas, which is easier but less reliable. First, the basal value of the parathyroid hormone concentration in serum is determined. Then one side of the neck is squeezed from the clavicle and along the trachea for 10–20 sec, and two hours later the same procedure is repeated on the other side. The parathyroid hormone concentration is checked in two further serum samples 1 min after the squeeze on each side. On the side of the parathyroid adenoma, there is usually an increase in the parathyroid hormone concentration, while on the other side the parathyroid hormone concentration does not vary from the basal value.

Recent experience suggests that cervical thermography may be a very useful method of localizing parathyroid disease and of differentiating parathyroid hyperplasia from adenomatous disease (SAMUELS, 1972).

7. Differential Diagnosis

Typical primary hyperparathyroidism is not difficult to diagnose. It is more difficult to evaluate poorly defined symptoms, noncharacteristic generalized disorders or gastrointestinal symptoms, and mental changes. It has already been stressed that it is essential that primary hyperparathyroidism be considered in the presence of symptoms of this kind.

Other diseases which have symptoms suggestive of hyperparathyroidism must also be taken into account. This applies mainly to nephrolithiasis, but also to certain skeletal conditions or radiological changes in the skeleton, nephrocalcinosis, and finally to idiopathic hypercalciuria. The single most important measurements are the determination of the serum calcium and the immunoreactive parathyroid hormone concentration.

a) Differential Diagnosis of Skeletal Involvement

The radiological findings in fully developed generalized osteitis fibrosa cystica are typical in primary hyperparathyroidism, which can hardly be confused with other skeletal disorders. The distinction from other metabolic skeletal disturbances can, however, be more difficult in milder forms and in the initial stages. This is true of renal osteopathy due to secondary hyperparathyroidism, and of the osteomalacia and the osteoporosis to a lesser extent, as well as of other nongeneralized skeletal disturbances.

α) Generalized Osteitis Fibrosa Cystica of Secondary Hyperparathyroidism

This has been observed particularly in chronic uremic renal diseases, in intestinal malabsorption, in vitamin-D deficiency of rickets in infancy, and in a very few cases of pseudohypoparathyroidism. The pathogenesis, the clinical symptoms which patients rarely complain of, the histology, biochemistry and radiological findings corresponding to those in primary hyperparathyroidism are discussed below (p. 936f.). The differential diagnosis is based on the chemical findings in the blood and on discovery of the underlying condition.

β) Rickets and Osteomalacia

These two skeletal conditions are also generalized and are characterized by the almost pathognomonic Looser's zones. Subperiosteal resorption is rarely observed (Figs. 26 and 27, p. 913f.). Acroosteolysis, bone cysts and osteoclastomas hardly ever develop. A reliable diagnosis can be made on radiological examination in many cases, based on these characteristic signs. The differentiation between renal osteopathy and intestinal malabsorption can be more difficult, since they are both mixed osteitic-osteomalacic diseases (p. 936f.). The findings in the bone biopsy, the hypocalcemia, hypophosphatemia and the elevated serum phosphatase are important for the diagnosis of osteomalacia.

γ) Osteoporosis

Radiological examination allows differentiation between this generalized skeletal affection and primary hyperparathyroidism: predilection for the vertebral column, pelvis, ribs and skull, slight involvement of the long bones, absence of subperiosteal resorption (on the contrary: well-maintained, sharply defined cortex), absence of bone cysts and osteoclastomas. Primary hyperparathyroidism mainly affects the 40–60 age group, whereas the age distribution is quite different for osteoporosis: post-menopausal osteoporosis, 5–8 years after the menopause, senile osteoporosis in the male sex in the 7th or 8th decade, idiopathic osteoporosis in the male sex (idiopathic hypercalciuria is discussed on p. 929) in the 3rd and 4th decades. The main symptoms in osteoporosis are lumbosacral pains and spontaneous fractures at typical sites (vertebral bodies, neck of femur, distal end of radius, proximal end of humerus). The

calcium, phosphate and alkaline phosphatase levels in the blood are normal in osteoporosis.

δ) Osteitis Deformans (Paget's Disease) and Polyosteitic, Fibrous Dysplasia (Jaffe-Lichtenstein Disease)

These conditions are monoosteitic or polyosteitic disorders focal but never generalized skeletal diseases. The pseudocystic changes can arise in a single place in fibrous dysplasia. When multiple pseudocysts are present, they are unilateral or segmental. The difficulties encountered in differentiating the skull in Paget's disease from the osteosclerotic skull in primary hyperparathyroidism have already been mentioned on p. 916. Alkaline phosphatase is discussed on p. 940.

ε) Multiple Myeloma

The diffuse infiltrative form of this disease sometimes simulates generalized metabolic atrophy of the bones, but the osteolytic foci of the myeloma can easily be mistaken for bone cysts or osteoclastomas. The possibility of a myeloma should always be considered in every case of a skeletal disease in advanced age. In every unexplained skeletal disorder, routine X-rays of the skull, thorax, the thoracic and lumbar vertebrae, pelvis and hand should be taken in addition to those of the affected part of the skeleton. Clinically silent lesions can thus be detected, e.g. osteolytic foci in the skull in multiple myeloma. The hematological findings and the serum proteins on electrophoresis are decisive factors in the diagnosis of multiple myeloma. The serum calcium is often elevated and rarely lower than normal; serum phosphate is normal, rarely elevated, and exceptionally reduced; the alkaline phosphatase is often normal.

ζ) Metastatic Skeletal Carcinomatosis

This condition is found particularly in carcinoma of the prostate, breast, thyroid, kidney and bronchus. Carcinomas of the gastrointestinal tract do not metastasize to the skeleton, with the exception of carcinoma of the rectum. As in multiple myelomas routine X-rays of the entire skeleton are important. Changes in the calcium and phosphate metabolism are often found in carcinoma of the prostate and of the breast (p. 927) but rarely in other types of carcinoma. In carcinoma of the prostate, the preferred localization is the pelvis. The X-ray findings can be similar to those in Paget's disease but in contrast to this disease, the osteoplastic metastases often extend beyond the cortical margin and can be seen on X-rays as-penetrations into the surrounding tissues. Another feature of Paget's disease with involvement of the pelvis is that the sacrum is practically always a site of lesions. This rarely the case in other similar skeletal disorders.

η) Solitary Bone Cysts and Giant Cell Tumors of Bones

These are circumscribed skeletal lesions. The biochemical findings are always normal. Confusion with the epulis does occur, but can be avoided if the normal biochemical findings are taken into account.

b) Differential Diagnosis of Nephrocalcinosis

Discrete depositions of calcium in the renal pyramids are quite often found on histological examination. They are of no clinical importance and cannot be detected on radiological examination. Fully developed nephrocalcinosis can be recognized even on a straight abdominal X-ray, and even better on a tomogram (Fig. 33, p. 918). Apart from primary hyperparathyroidism, vitamin-D intoxication, tubular acidosis and every form of hypercalcemia can also lead to severe nephrocalcinosis. Milder forms of nephrocalcinosis can also occur in the Franconi's syndrome, occasionally in idiopathic hypercalciuria, and in rare cases of poisoning with uranium, mercury, and oxalates. It also occurs occasionally in interstitial nephritis. The X-ray findings may sometimes be confused with depots of thorotrast, a contrast medium no longer in use, which was formely used for the retrograde pyelogram.

c) Differential Diagnosis of Hypercalcemia

Hypercalcemia is the most important diagnostic criterion for primary hyperparathyroidism, but it is not pathognomonic of this condition. It has been observed in a series of different illnesses, which are listed roughly in order of frequency below. Several hypercalcemic factors can be combined in individual cases. An example of this type is immobilization, which by itself only exceptionally causes hypercalcemia, but which in combination with metastatic skeletal carcinomatosis, Paget's disease, bone fractures, orthopedic surgery, poliomyelitis, and vitamin-D therapy sometimes produces hypercalcemia. In principle, analogous combinations of all the factors listed are possible, and some are very well known (p. 923).

α) Causes of Hypercalcemia

1. Malignant tumors, with or without skeletal metastases;
2. Primary hyperparathyroidism;
3. Hyperthyroidism;
4. Sarcoidosis;
5. D-hypervitaminosis;
6. Multiple myeloma and other hemoblastoses;
7. Estrogen and androgen treatment in malignant tumors;
8. Immobilization, e.g. after bone fracture, orthopedic surgery;
9. Paget's disease;
10. Primary bone tumors;
11. Milk-alkali syndrome;
12. Addison's disease, withdrawal of adrenocortical steroids;
13. Idiopathic hypercalcemia of childhood;
14. Thiazide diuretics.

β) Malignant Tumors

Hypercalcemia found incidentally, is due to a malignant tumor approximately as often as to primary hyperparathyroidism. Carcinoma of the breast is the most common malignant tumor giving rise to hypercalcemia, particularly during treatment with estrogens and androgens. However, other malignant tumors, such as multiple myeloma, hypernephroma, carcinoma of the prostate, bronchus, thyroid and ovary, neuroblastoma, and various sarcomas can also cause hypercalcemia. Hypercalcemia also occurs in leukemia during childhood. The serum calcium is considerably elevated in many cases, whereas the serum phosphate is only slightly elevated, normal, or reduced. Hypercalcemia is usually caused by the skeletal metastases, but occasionally it can also occur in the absence of skeletal metastases, and can disappear after removal of the malignant tumor. This is seen particularly with hypernephromas and bronchial carcinomas, which sometimes secrete parathyroid hormone (RIGGS, 1971). SHERWOOD was able to demonstrate by radioimmunological methods that parathyroid hormone levels can be elevated in serum and in tumor tissue in patients with hypercalcemia caused by various tumors. Very rarely, osteoplastic metastases of carcinomas lead to hypocalcemia (p. 897; Table 1, p. 849). Osteoclastic resorption zones are seen around skeletal metastases of paraneoplastic parathyroid hormone-producing tumors, as described by BARNICOT in 1941 with a parathyroid gland transplantation onto the surface of the skeleton (p. 874) (MELVIN, 1971).

γ) Hyperthyroidism

Hypercalciuria is almost a frequent symptom of hyperthyroidism, whereas slight hypercalcemia is found only occasionally, and marked hypercalcemia only exeptionally. An injection of parathyroid hormone produces more pronounced hypercalcemia in hyperthyroid patients than in healthy subjects, due to the summation of two hypercalcemic factors (HARRISON, 1964). Marked hypercalcemia can be expected when primary hyperparathyroidism and hyperthyroidism occur at the same time. The differential diagnosis in this case is not easy and must be based on determination of the parathyroid hormone concentration in the serum.

δ) Sarcoidosis

As in hyperthyroidism, hypercalciuria is often observed, whereas hypercalcemia is rare. Cortisone suppresses the hypercalcemia in sarcoidosis promptly and almost without exception. Patients with sarcoidosis are highly sensitive to vitamin D (danger of a hypercalcemic crisis). Sunlight alone can lead to hypercalcemia in sarcoidosis, which is still frequently mistaken for primary hyperparathyroidism. A diagnosis of primary hyperparathyroidism should thus not be made until sarcoidosis has been excluded. The situation is further complicated by the fact that there have been several reports of sarcoidosis occurring at the same time as primary hyperparathyroidism, proof of the latter condition being provided by the surgical removal of a parathyroid adenoma and radioimmunologic determination of elevated parathyroid hormone levels (p. 904).

ε) D-Hypervitaminosis

This condition was previously often observed in association with large doses of vitamin D used in the treatment of primary chronic polyarthritis, lupus vulgaris and sarcoidosis. To-day severe hypercalcemia with the full-blown picture of the hypercalcemic syndrome is encountered much less frequently, sometimes when repeated doses of vitamin D are given intermittently without sufficient indication, or with vitamin-D therapy in cases with elevated sensitivity to vitamin D. Every patient with an additional hypercalcemic factor must be considered as being oversensitive to vitamin D (p. 927). It has recently been demonstrated that hypersensitivity to vitamin D is possible not only in sarcoidosis but also in tuberculosis (SHAI, 1972). In these cases normal doses of vitamin D can produce severe hypercalcemia. A history of previous treatment with vitamin D or the more or less unnoticed intake

of vitamin D in food or as multivitamin is a deciding factor in the differential diagnosis between overdosage with vitamin D and primary hyperparathyroidism, due to the close similarity of the two clinical conditions.

Administration of average doses of vitamin D occasionally makes the diagnosis of primary hyperparathyroidism difficult. Since therapeutic doses of vitamin D remain active for some weeks or even months, the biochemical diagnosis of primary hyperparathyroidism is only possible when increased immunoreactive parathyroid hormone concentrations are measured in the serum. The differential diagnosis can also be made from the clinical features and the possible cause of the hypercalcemia. If this fails in cases of mild hypercalcemia, it is permissible to wait until the action of the vitamin D has disappeared. In cases of severe hypercalcemia, however, the procedure recommended for the treatment of the acute hyperparathyroidism must be used (p. 933).

ζ) Idiopathic Hypercalcemia in Childhood

It should not be too difficult to differentiate the various forms of this syndrome from primary hyperparathyroidism, since primary hyperparathyroidism has never yet been described in children less than two years old. Idiopathic hypercalcemia is also associated with typical facial changes, debility, and multiple changes in the large arteries (WILTSE, 1966), such as supravalvular aortic stenosis.

η) Milk-Alkali Syndrome (Burnett Syndrome)

The intake of alkaline powders together with large amounts of milk over some months for the treatment of gastric ulcer can lead to symptoms very similar to those seen in vitamin-D overdosage and in hyperparathyroidism. Extensive calcium depositions, band keratitis, and calcifications of the periarticular tissue occur. In addition, there is usually severe renal failure. The metabolic picture is characterized by hypercalcemia, hyperphosphatemia, and hypocalciuria, the last being an important diagnostic feature. The condition is usually easily distinguished from hyperparathyroidism by the behavior of the calcium in the urine and the phosphate in the blood, and also by the clinical history. Difficulties can arise, however, when renal failure develops in primary hyperparathyroidism due to nephrolithiasis or nephrocalcinosis. In a case of this kind, the metabolic picture can be similar to that in the milk-alkali syndrome. Further difficulty is encountered when a gastric or duodenal ulcer arises during the course of primary hyperparathyroidism and is then treated with milk and alkaline salts. The differentiation between the Burnett syndrome and hyperparathyroidism associated with the Burnett syndrome can then be very difficult. Immunoreactive parathyroid hormone should be determined in the serum. A diagnostic exploratory operation of the cervical organs is then indicated in doubtful cases with severe hypercalcemia refractory to therapy. The treatment for acute hyperparathyroidism should otherwise be followed (p. 933).

ϑ) Immobilization

It has already been mentioned that immobilization alone very rarely leads to hypercalcemia. However, hypercalcemia occurs more frequently when an additional, even minor, factor promoting calcium mobilization occurs. As well as the factors given in section α (p. 927), the following situations have also been implicated: bone fractures in young subjects, Paget's disease, bone metastases; previous long-term treatment with vitamin D for vitamin D-resistant rickets at the time of the immobilization, e.g. after an orthopedic operation.

ι) Addison's Disease

It has been known for some years that the Addisonian crisis can cause hypercalcemia. Chronic hypercalcemia in ADDISON'S disease is rare and occurs predominantly during childhood (PRADER). Mild hypercalcemia is found in about one third of untreated Addisonian patients. Hypercalcemia can also arise after total adrenalectomy and after sudden withdrawal of treatment with cortisone or ACTH.

κ) Paget's Disease

In the presence of extensive skeletal involvement, hypercalcemia can arise due to immobilization following a fracture. Previous treatment with vitamin D may even favor this complication. Hypercalcemia due to immobilization is uncommon in Paget's disease (GORDAN and LIÈVRE).

λ) Thiazide Diuretics

It has been known for some time that diuretics of the thiazide group almost always produce hypocalciuria by inhibition of the tubular reabsorption of sodium (amount of calcium excreted in the urine in 24 hours decreased by 30% or more, LICHTWITZ). The fact that the same diuretics can produce severe hypercalcemia

in the presence of a preexisting mild elevation of the serum calcium is less well known; it does, however, occur, but less frequently. VAN DER SLUYS VEER (1965) has observed this feature repeatedly in primary hyperparathyroidism. Thiazide diuretics are not known to produce an acute hypercalcemic syndrome by themselves. It is important to know about the mechanism of this type of hypercalcemia since dangerous hypercalcemia can be prevented by withdrawal of these drugs.

d) Differential Diagnosis of Hypercalciuria

Changes in the serum calcium level and in the amount of calcium excreted in the urine are generally parallel. There are two important exceptions to this rule: the milk-alkali syndrome, in which the hypercalcemia is associated with normo- or hypocalciuria, and multiple myeloma, in which hyper- normo- or hypocalciuria can be present. The absence of hypercalciuria is due to renal failure in both cases. Primary hyperparathyroidism also exceptionally runs a course without or with only mild hypercalciuria, due to the stimulation of the calcium reabsorption in the renal tubules by parathyroid hormone, or to renal failure. In spite of this, hypercalciuria is an important feature in the diagnosis of primary hyperparathyroidism (p. 941). Since hypercalciuria has been observed in numerous clinical conditions and often in association with nephrolithiasis, as in primary hyperparathyroidism, however, the differential diagnosis must be made in every case of hypercalciuria. The table below gives the various forms of hypercalciuria with or without hypercalcemia.

Hypercalciuria resulting from hypercalcemia is due to elevated glomerular filtration of calcium, while hypercalciuria in the absence of hypercalcemia is due to impaired tubular reabsorption of calcium (p. 852). In some cases, e.g. after the administration of high doses of vitamin D, both factors can function at the same time, producing an additive effect.

Table 6

Causes of hypercalciuria

A. Hypercalciuria resulting from hypercalcemia
1. Malignant tumors with or without skeletal metastases
2. Primary hyperparathyroidism
3. Multiple myeloma and other hemoblastoses
4. Estrogen and androgen treatment of malignant tumors
5. Idiopathic hypercalcemia of childhood
6. Addison's disease

B. Hypercalciuria with facultative hypercalcemia
1. Hyperthyroidism
2. Sarcoidosis
3. Vitamin D overdosage
4. Immobilization
5. Bone fracture, orthopedic operation
6. Paget's disease
7. Primary bone tumor

C. Hypercalciuria with normocalcemia
1. Idiopathic hypercalciuria
2. Acute osteoporosis
3. Cushing's syndrome
4. Cortisone therapy
5. Vitamin-D therapy (isolated cases)
6. Tubular acidosis (isolated cases)
7. Pyelonephritis

D. Hypercalciuria with hypocalcemia
1. Vitamin-D therapy for hypocalcemic conditions occasionally early in the treatment
2. Tubular acidosis
3. Pyelonephritis

Causes of hypocalciuria

E. Hypocalciuria with hypercalcemia
1. Milk-alkali syndrome
2. Multiple myeloma

F. Hypocalciuria with normocalcemia
1. Multiple myeloma
2. Hypothyroidism
3. Growth
4. Vitamin-D deficiency
5. Intestinal malabsorption

G. Hypocalciuria with hypocalcemia
1. Hypoparathyroidism
2. Other forms of hypocalcemia

H. Hypocalciuria independent of the concentration of the serum calcium
1. Renal insufficiency

Idiopathic Hypercalciuria. It is often difficult to differentiate this condition from primary hyperparathyroidism. Idiopathic hypercalciuria is found in about 10% of patients with renal stones (MELICK). It is characterized by hypercalciuria, normocalcemia, and sometimes mild hypophosphatemia. The following clinical features are worth mentioning: recurrent nephrolithiasis (oxalate, phosphate or mixed oxalate and phosphate stones), infrequent idiopathic osteoporosis (beginning in the 3rd or 4th decade, a rare type of osteoporosis of the male sex), a strong predominance in the male sex. Idiopathic hypercalciuria can also occasionally be observed alone, in the absence of nephrolithiasis or of skeletal disturbances.

Idiopathic hypercalciuria with recurrent nephrolithiasis is the first condition to be considered in the differential diagnosis of hyperparathyroidism with nephrolithiasis. All forms of symptomatic hypercalciuria must be excluded initially in both these conditions (Table 6, p. 929). It is also advisable to estimate the immunoreactive parathyroid concentration and the serum calcium at weekly intervals for some time. In some cases, the parathyroid suppression test

after HOWARD, KYLE and HAAS (p. 943), or the parathyroid hormone test after BECKER (p. 944) can be helpful. The cortisone test after DENT is not helpful in cases of mild hypercalcemia. Some authors (MATHER, 1953; WILLS, 1969) suggest the existence of rare cases of primary hyperparathyroidism with bilateral recurrent nephrolithiasis as an isolated clinical symptom with normal laboratory findings. Since differentiation from idiopathic hypercalciuria would not be possible in these circumstances, they recommend exploratory revision of the neck if the clinical findings are alarming despite the laboratory findings. Whether this concept is correct will be demonstrated when a large number of well-documented cases with reliable radioimmunologic measurements of the parathyroid hormone concentration and perfect parathyroid histology is reported in the literature. Thiazide or phosphate can be used in the treatment of idiopathic hypercalcemia, since they reduce the calcium excretion by the kidneys (YENDT, 1966). Thiazides may cause hypercalcemia mainly in cases of preexisting mild hypercalcemia (cf. section on the differential diagnosis of hypercalcemia, p. 927). Primary hyperparathyroidism can become evident following the treatment of idiopathic hypercalcemia with chlorothiazides (DUARTE, 1971; ADAMS, 1971). Furosemides are the only diuretics which lower serum calcium.

8. Prognosis

The prognosis of primary hyperparathyroidism is determined by the extent of the irreversible limitation of renal function. Early diagnosis and treatment are therefore desirable. It is questionable, however, whether the sinister picture of uremia in the terminal stages of nephrocalcinosis or bilateral pyonephritis is applicable to most cases of hyperparathyroidism, or only to a small number of patients. We are inclined to accept the second, more optimistic, prognosis. We know of exceptional cases of primary hyperparathyroidism which have been cured spontaneously due to spontaneous necrosis of the parathyroid adenoma (p. 906). Furthermore, statistics show that primary hyperparathyroidism is a relatively common condition (5% of all cases of renal calculus according to MELICK, p. 919, 1.2% of all hospital patients according to BOONSTRA, p. 905). Only a small proportion of these cases are diagnosed, treated surgically, and cured in most hospitals all over the world. If, however, only half the unknown cases have a fatal outcome because of nephrolithiasis or nephrocalcinosis such illnesses should be observed frequently, clinically and at autopsy. We conclude that the course of the illness is more favorable than assumed and may extend over years, and that spontaneous healing of primary hyperparathyroidism occurs more frequently than was thought earlier, due to spontaneous necrosis or other unknown causes. It is impossible to give even a vague figure for the frequency of the favorable outcome.

9. Therapy

Surgical removal of the parathyroid adenoma is almost the treatment of choice for primary hyperparathyroidism (the medical treatment of hypercalcemia is described on p. 932 and p. 933). Radiation of the parathyroid adenoma has proved ineffective. A high serum calcium must be lowered preoperatively if possible.

The removal of a parathyroid adenoma requires an experienced surgeon, and patients should be referred to an adequate clinic. ALBRIGHT has expressed the opinion that the most important step in obtaining successful results in the treatment of hyperparathyroidism is the choice of the right surgeon. It is particularly dangerous when a surgeon with little experience decides "to have a look". Two results are then possible; either the removal of the parathyroid adenoma is easy and the intervention ends successfully even when performed by an inexperienced surgeon, or location of the adenoma is exceptionally difficult and the surgeon with little experience does not succeed in locating it and causes a large number of fibrous adhesions which greatly handicap a second surgical procedure. As is typical in surgery, success is only seen after the surgeon has had experience with 50 of the same sort of cases.

This very pointed statement from ALBRIGHT describes a delicate problem in hard words. It may be absolutely justified in large surgical centers, but a compromise must be made in smaller centers. In general, it is recommended that parathyroid operations should be left to the few surgeons who have had the opportunity to perform these operations frequently. In any case, it is essential for the surgeon to study the literature, even if he is not a specialist in this field. We mention here the works published by CHURCHILL and COPE and in particular the exceptionally thorough and critical monograph by BLACK.

A few fundamental points about the surgical technique will be quoted from BLACK's work at thispoint. The possibilities and limitations of preoperative localization of the adenoma have been discussed on p. 924. The surgeon must be convinced of the correctness of the

diagnosis before the operation. He must have sufficient time at his disposal for the procedure, since though the parathyroid adenoma can become visible in the field of operation within a few minutes, it can take several hours of careful dissection to find it. If an adenoma cannot be found, the surgeon must make use of the intactness of the operation field in the first operation to inspect the positions of the normal parathyroids, to mark them, and if possible to perform a biopsy. The parathyroid biopsy must be performed with the greatest care. The presence of a pathologist experienced in the histology of the parathyroids is therefore indispensable throughout the operation.

The upper parathyroids should be looked for in the following positions: on the posterior surface of the thyroid gland, in the upper and posterior parts of the mediastinum between the trachea and esophagus, and in the connective tissue around the large vessels. The lower parathyroids must be searched for at or behind the lower pole of the thyroid gland, in the upper posterior mediastinum, and in the anterior region of the mediastinum. It is important to expose the way to the lower thyroid pole by ligating the inferior thyroid vein or the ima vein when necessary. If the parathyroid adenoma is located some distance from the thyroid, there is almost always a distinct vascular stalk arising from the inferior thyroid artery and leading to the parathyroid adenoma. Some adenomas of the anterior mediastinum are an exception to this, when they develop in a primarily abnormally located parathyroid. In this case, there is no vascular stalk running up from below (cf. p. 846). The size of parathyroid adenoma expected can only be prophesied with reservations. Statistics, however, show that the severity of the hypercalcemia and the increase in parathyroid hormone concentrations are in direct proportion to the weight of the adenoma. The adenomas in cases of hyperparathyroidism with skeletal involvement are usually larger than in cases with only renal complications. The variation between these two rules, however, is too wide for the surgeon to be guided by them during the operation. Parathyroid adenomas are small in some cases, e.g. only 100–250 mg. BLACK indicates that the small adenoma can present more difficulties to the surgeon than an abnormal location, but he is more optimistic than other surgeons about the abnormal position. Among 200 operations performed for parathyroid adenomas at the Mayo Clinic, BLACK only had to search for the adenoma in the anterior mediastinum after dividing the sternum in three cases. He performs the operation under local anesthesia supplemented with nitrous oxide. Other surgeons prefer a general anesthetic. Intubation anesthesia without curare is used at the Urology Clinic at Zurich University Hospital (MAYOR). Local anesthesia, especially with adrenaline added to the anesthetic fluid, has been rejected because of the danger of ventricular fibrillation. All cardiac glycosides are forbidden in hyperparathyroid patients for the same reason (p. 923). The Kocher collar incision is made in the usual way, the thyroid gland is exposed, and the parathyroids are searched for in turn. When a parathyroid adenoma is discovered during this process, it is totally removed. Partial resections used to be carried out because of the danger of the postoperative tetany but are no longer necessary. The removal of a normal parathyroid instead of an unfound parathyroid adenoma should never be permitted (p. 890), although it is not always easy to differentiate macroscopically between hyperplastic and normal glands. If three or four normal parathyroids are located, and still no adenoma is found, intrathyroidal localization of the adenoma must be considered. Usually an adenoma of this type is enclosed in the lower pole of one lobe of the thyroid and can be recognized by a slight bulge in the thyroid at this site. If there is the slightest indication of an intrathyroidal adenoma, examination of the cervical organs must be followed by partial resection of the thyroid. MAYOR even recommends subtotal thyroidectomy, leaving the upper part of the pyramid median lobe intact in cases where the exploration of the cervical organs is negative. Inspection of the anterior mediastinum is carried out at a second operation, not less than 1–3 months after the first. In the interval it would become obvious whether the hyperparathyroidism persists, or whether it has been cured by unrecognized removal of the adenoma or by ligation of the vascular supply to the parathyroid adenoma. The calcium and phosphorus abnormalities then return to the normal state. Before the second exploratory operation is undertaken, immunoreactive parathyroid hormone concentrations in the serum should be measured and special attention paid to a possible overdosage with vitamin D or other causes of hypercalcemia. The anterior mediastinum is inspected through a division of the sternum from the manubrium to the xyphoid process, or through a transverse incision opening the thorax in the 4th intercostal space and dividing the sternum transversely. If no adenoma is found after careful dissection of the whole region, the thymus must then be removed. This is done as an ultimate procedure, after the arch of the aorta with its branches, the pulmonary artery,

the left anonymous vein and the surface of the pericardium have been exposed. If a procedure involving division of the sternum is essential, the removal of a bone biopsy must never be neglected.

A special procedure is recommended for cases of primary hyperplasia of the water-clear cells. The surgeon recognizes this condition from the gross enlargement of all four parathyroids. In some cases, one gland can be much larger than the others, simulating a solitary adenoma. In these cases the diagnosis is possible only from the biopsy, which shows the same characteristic picture in all four glands (p. 907). If the diagnosis is confirmed, all the parathyroid tissue except a small well-vascularized tissue residue of about 100–200 mg must be radically excised. It seems that it is not always easy to decide what is a well-vascularized piece of tissue. BLACK observed postoperative tetany several times among 15 of his own cases.

The problem of the risk of recurrences of multiple parathyroid adenomas (primary hyperplasia of the chief cells) has already been discussed above. Again no more than 100–200 mg of well-vascularized tissue should be left behind. BLACK recommends marking the residual tissue with a black suture, because of the danger of recurrences entailing a second operation (p. 908).

Cases in which exploration of the cervical organs and mediastinotomy yield no positive findings, and those in which hyperparathyroidism persists after removal of a parathyroid adenoma, present a difficult problem. In such cases, the diagnosis must be minutely revised, taking all the possibilities into consideration in the differential diagnosis. If the diagnosis of hyperparathyroidism is confirmed, a surgeon with wide experience in the field of parathyroid surgery should be consulted for a second operation. At this point we should like to recall the classic case of Captain Martell; a parathyroid adenoma in the anterior mediastinum was removed by CHURCHILL at the 7th operation (COPE, 1966).

The first object of any treatment adopted in the interval before a second operation must be the prevention of renal complications and of acute, excessive hypercalcemic hyperparathyroidism, because these complications are dangerous. The first and most important measure is to encourage the intake of very large amounts of fluid. This is best done by distributing the fluid over the 24 hours, e.g. by taking 600–1000 ml approximately every 6 hours, waking up once during the night as well. When excessive hypercalcemia seems imminent, the phosphate infusion which is always ready or furosemide treatment should be started without delay (p. 933).

Patients who refuse surgery or are not cured by an operation must be treated with intravenous infusions of phosphates given at regular intervals. Oral phosphate treatment can be tried in less severe cases (p. 933). There is still no experience of the results achieved with long-term calcitonin treatment. In cases of hyperparathyroidism where the diagnosis is uncertain, an attempt to reduce the elevated calcium concentration with cortisone is worthwhile.

10. Postoperative Treatment

After successful removal of the adenoma, postoperative oliguria, and an immediate reduction of the urinary excretion of calcium, magnesium and of phosphorus are usually observed at first (GOLDSMITH, 1966). The values for serum calcium and phosphorus return to normal ranges within two to three days, but it is often much longer before the hypophosphatemia becomes normal; weeks and months can elapse before it occurs, or it may even fail to occur, without causing the success to be evaluated as incomplete. If the operation is unsuccessful, the serum calcium and the other metabolic changes fail to become normal or are only partially normalized.

Postoperative tetany develops in a few cases after successful removal of the adenoma. Hyperparathyroidism without apparent skeletal involvement is rarely associated with this complication, and then only mildly, but severe tetany lasting for weeks can develop in cases with extensive skeletal involvement and elevated serum phosphatase. The etiology of the two forms of tetany is not the same. The mild, transient form associated with the extra-osseous hyperparathyroidism is probably explained by parathyroid insufficiency due to atrophy of the nonadenomatous glands remaining in the organism. Severe postoperative tetany of hyperparathyroidism with skeletal involvement is due to the excessive recalcification of the bones in addition to parathyroid insufficiency. The skeleton is deprived of mineral substances and shows a great avidity for calcium, phosphorus, and magnesium after the parathyroid adenoma has been removed. Serum calcium, magnesium and phosphorus fall to extremely low levels. The more severe the skeletal changes are with high preoperative values of the serum phosphatase, the more severe this form of the postoperative "recalcification tetany" can be.

Treatment of severe postoperative tetany should not be postponed until the full tetany syndrome develops. Tetany must be expected postoperatively in patients with a high serum phosphatase. The serum calcium must be estimated several times on the first day. Intravenous calcium and vitamin D are then given according to the method of treatment described in p. 902. As soon as oral feeding is possible, the patient receives 30–50 g calcium lactate or calcium gluconate and 50–100 mg magnesium oxidate p.o. The dose of calcium is later reduced to 10–15 g while the magnesium dose remains unaltered. When the serum magnesium falls very low, down to 0.8 mEq/liter or less, magnesium can be given intravenously in a dose of 2 mEq/kg body weight over four hours, or up to 4 mEq magnesium/kg body weight daily. This dose can later be reduced to 0.25–0.5 mEq magnesium/kg body weight/day (MACINTYRE, 1967). Prophylactic use of intravenous vitamin D immediately after the operation in cases with an elevated phosphatase is not without danger. Even the surgeon experienced in parathyroid surgery can sometimes find only one of several parathyroid adenomas which may be present, with the result that the metabolic changes of primary hyperparathyroidism persist; in a case of this type, a single large dose of vitamin D could precipitate a parathyroid crisis. If there is still a tendency towards hypocalcemia after the first acute symptoms have subsided, vitamin D in a dose of 1.25–2.5 mg (= 50000–100000 units) or dihydrotachysterol (AT 10) in a dose of 8–10 drops can be given orally each day. It may take months for the skeleton to become recalcified. Until then, treatment with oral vitamin D combined with calcium and magnesium salts should be continued.

The bone pains usually disappear rapidly after successful operations, often even within hours. The recalcification of the skeleton, however, takes several months, during which there is still a higher predisposition to fractures.

Paresis of the recurrent laryngeal nerve developing with postoperative tetany is dangerous. An intravenous calcium injection should therefore always be kept ready. In doubtful cases, tracheotomy should not be postponed too long.

Postoperative psychosis of the acute exogenous reaction type has been dealt with above (p. 922).

11. Treatment of the Parathyroid Crisis

Measures must be taken at once to ensure adequate fluid intake at the first signs of an acute hypercalcemic crisis (p. 923). The administration of calcium and vitamin D in any form is to be avoided, as is treatment with thiazide diuretics. Administration of phosphates by the i.v. route has proved the most effective treatment. They should be given as recommended by GOLDSMITH: 1 liter of a sodium and potassium phosphate buffer solution at pH 7.4, containing 0.081 mol Na_2HPO_4 + 0.019 mol KH_2PO_4, to be given over 6–20 hours depending on the renal function. In severe hypercalcemia the renal function is rarely normal and extreme caution must be applied in the intravenous administration of phosphates. The most important element in the treatment of a parathyroid crisis is a high fluid intake, which may be combined with diuretics such as furosemide, which increase urinary calcium excretion (p. 851). The serum calcium begins to fall even during the phosphate infusion and sometimes reaches normal limits on the first day. The infusion can be repeated every 24 hours or whenever the serum calcium begins to rise again. The duration of action of the phosphorus administered varies widely. The same hypocalcemic effect can be achieved with oral phosphorus in less severe states. WILSON and YENDT recommend the following mixture for the oral treatment: 2.1 g $NaH_2PO_4 \cdot H_2O$ + 30.0 g $Na_2HPO_4 \cdot 12H_2O$ in one liter of calcium-free water. 100 ml of the solution contains 300 mg phosphorus. The daily dose is 300–600 ml or 0.9–1.8 g phosphorus, divided into 4 doses. This has recently been produced commercially in the form of sugar-coated tablets, under the name of "Reducto". In the recent past, there have been objections to the treatment with i.v. phosphates (BREUER and SCHACKNEY, 1967), but there have been few cases of death occurring during or after the infusion among the numerous cases benefited by this method (KISTLER), and the cause of death was not fully clarified in any of them. These authors have recommended that i.v. phosphates not be given in renal failure. The dose can be reduced to 500–250 ml and the phosphate given more slowly. The serum calcium, phosphorus, creatinine, and urea have to be monitored at short intervals. Caution is also indicated in hypercalcemia of multiple myeloma, since renal failure is often already present. In summary, it appears that intravenous administration of phosphates is the best available treatment of acute hypercalcemia at present when carried out correctly. The most important treatment in a parathyroid crisis is a high fluid intake. This may be combined with i.v. phosphate or diuretics, such as furosemide, which increase urinary calcium excretion. Furosemide (Lasix) can be given after the patient has been hydrated with 1–2 liters

of saline. The intravenous dose amounts to 80–100 mg every 1–2 hours leading to an average daily dose of 1240 mg (160–3200 mg). This results in an increased urinary excretion of calcium, sodium, and water. It is therefore essential to check the water balance (intake and excretion) every hour, to determine the concentrations of calcium, sodium, potassium, and magnesium in the serum, and if necessary replace the sodium, potassium, and magnesium lost in the urine (SUKI, 1970). A favorable hypocalcemic effect has also been observed with sodium sulfate ions, but very large amounts must be given, which can endanger the circulation (an isotonic solution with 116.7 g sodium sulfate in 3 liters water over 9 hours, after CHAKMAKIJAN). In desperate cases, peritoneal dialysis or hemodialysis can be used. Whether calcitonin (COPE and FOSTER) or mithramycin (SINGER) will solve the problem of the treatment of hypercalcemia is not known. Trials have shown only partial lowering of the elevated blood levels of calcium of different etiologies following calcitonin (HASS, 1967). The cytostatic antibiotic mithramycin (MITHRACIN and PFIZER) has recently been introduced to lower serum calcium especially in tumor hypercalcemia or if all other therapeutic measures have failed. The drug has been used in the treatment of metabolically active Paget's disease, despite possible side effects. If the diagnosis of primary hyperparathyroidism has been confirmed or is very likely, no time should be lost in operating immediately after rehydration and reduction of the hypercalcemia.

H. Secondary Hyperparathyroidism

M. WERNLY

1. Definition

In secondary hyperparathyroidism parathyroid hormone secretion is increased secondary to various stimuli such as a lowered calcium or magnesium concentration in the serum. Synthesis and release of parathyroid hormone into the blood are increased and there is generalized hyperplasia of the parathyroid glands.

2. Morbid Anatomy and Histology

The generalized hyperplasia of the parathyroids in secondary hyperparathyroidism involves all four glands and is termed *secondary* parathyroid hyperplasia because of the special etiological factors and because it contrasts with the primary hyperplasia of the chief cells (p. 909) and the water-clear cells (p. 907). A diffuse and a microadenomatous form are differentiated histologically.

The term microadenomatous hyperplasia implies only the morphological picture and not the functional state of the glands. Microadenomas are found in morphologically indistinguishable forms in primary as well as in secondary hyperparathyroidism.

1. Diffuse hyperplasia is characterized by uniform, compact enlargement of all four glands, in which nearly all the parenchymal cells are converted into large chief cells or into small water-clear cells. There are few fat cells. The structure is compact and homogeneous. The weights of the different glands can vary widely (cf. p. 848).

2. Microadenomatous hyperplasia is due to a focal proliferation of the smaller and middle-sized chief cells. Only a few large chief cells and water-clear cells are found. Multiple proliferations arise in all sections of the glands and grow to form microadenomas of different sizes, while the original glandular lobules become compressed to narrow cellular bands during the process. There are no fat cells in the microadenomatous regions, though they may be present in the original lobules. The weight of the different glands varies widely. The total weight of the glands is higher in the microadenomatous form than in diffuse hyperplasia.

It is not possible to differentiate between the two forms of hyperplasia etiologically or functionally. Clinical data indicate that microadenomatous hyperplasia is associated with a more intensive increase in the hormonal activity than the diffuse form. The difficulties in differentiating secondary microadenomatous hyperplasia from primary hyperplasia of the chief cells have been discussed above (p. 908).

3. Incidence

As parathyroid hormone in the serum is not estimated routinely, the conditions leading to secondary hyperparathyroidism remain insufficiently explained. We can only suspect a secondary hyperparathyroidism where patho-anatomical evidence of a secondary parathyroid hyperplasia is found. This unsatisfactory criterion has now been replaced by the demonstration of increased levels of parathyroid hormone in the serum in renal insufficiency, intestinal malabsorption, infantile rickets and pseudohyperparathyroidism, which are the most common forms leading to secondary hyperplasia (BERSON, 1966; POTTS, 1969; LEGUIN, 1970; FOURNIER, 1971; HACKENS, 1972). Clinical tests, such as the measurement of the phosphate

clearance, or preferably the TmP, the measurement of the urinary hydroxyproline excretion and the morphometric evaluation of bone biopsies are valuable indications of an increased parathyroid hormone secretion in parathyroid hyperplasia. In conclusion, there is a good correlation between the pathologic-anatomical evidence of parathyroid hyperplasia and increased parathyroid hormone concentration in the serum in secondary hyperparathyroidism.

Parathyroid hyperplasia is observed in chronic renal failure and in vitamin-D deficiency. It is seen considerably less frequently and in only a small proportion of cases of leukemia, multiple myeloma, skeletal metastases, Paget's disease, liver cirrhosis and certain gangrenous infections (WERNLY, 1946).

Secondary hyperparathyroidism can also be clinically diagnosed in the presence of a typical osteopathy (subperiosteal resorption), or metabolically or morphometrically from elevated bone resorption. Clinically, secondary hyperparathyroidism can be demonstrated in rare cases of pseudohypoparathyroidism. Furthermore, increased bone destruction can be demonstrated in certain forms of osteoporosis with the aid of bone biopsies and of kinetic methods. It is not certain whether an increased release of parathyroid hormone is associated with this process.

4. Etiology

In the majority of cases, parathyroid hormone secretion is stimulated by a lowered serum calcium concentration lasting for months or years in chronic uremic renal disorders and in intestinal malabsorption. The importance of the hypocalcemia for the stimulation of the parathyroid glands has been demonstrated experimentally; perfusion experiments with thyroid-parathyroid preparations, using extracorporeal circulation, have shown that hypocalcemic perfusing blood (EDTA-hypocalcemia) results in the release of parathyroid hormone, whereas the blood draining away has hypercalcemic effects (COPP) and a radioimmunologically elevated parathyroid hormone concentration (CARE). Recently, BERSON and YALOW have even demonstrated radioimmunologically a rise in the parathyroid hormone level after EDTA- induced hypocalcemia.

Hypomagnesemia can also cause secondary hyperparathyroidism and hypermagnesemia can suppress parathyroid hormone secretion. Using parathyroid explants *in vitro*, SHERWOOD (1971) has demonstrated that the parathyroid hormone secretion is inversely proportional to the magnesium concentration in the incubation medium. Furthermore, CARE was able to suppress the parathyroid hormone secretion *in vitro* by perfusing the parathyroids with hypermagnesemic blood. Clinically, little is known about the importance of magnesium in secondary hyperparathyroidism. It is possible that hypomagnesia is the cause of the secondary parathyroid hyperplasia observed in liver cirrhosis.

It is still undecided whether there are other factors besides hypocalcemia and hypomagnesemia which cause secondary hyperparathyroidism. We cannot entirely exclude this possibility because of the only very mild hypocalcemia encountered in certain cases of chronic renal insufficiency and intestinal malabsorption, and because of the difficulty in explaining certain uncommon forms of parathyroid hyperplasia (in leukemia, multiple myeloma, metastatic skeletal carcinomatosis, Paget's disease, liver cirrhosis, and gangrenous infections). It is certain, however, that a rise in the serum phosphate alone does not lead to secondary hyperparathyroidism, because it is always associated with a lowering of the serum ionized calcium concentration (FISCHER, 1972). It appears, however, that hypocalcemia is the most important stimulus, because if phosphate and calcium are given together no increase in parathyroid hormone concentration in the serum is seen (SHERWOOD, 1968; REISS, 1971).

5. Pathophysiology

Primary hyperparathyroidism is a disease of the parathyroids, whereas secondary hyperparathyroidism is a combination of a primary disease and of a parathyroid disorder.

Secondary hyperparathyroidism due to vitamin-D deficiency is caused by the hypocalcemia found in all forms of osteomalacia. Accordingly, there is almost always parathyroid hyperplasia (WERNLY, 1946), elevated phosphate clearance, an increased urinary excretion of hydroxyproline and an increase in the number of osteoclasts, reflecting the raised parathyroid activity (NORDIN, 1956; CRABBÉ, 1965; FISCHER, 1968). The increased release of parathyroid hormone into the blood corrects the hypocalcemia, depending on the severity of the vitamin-D deficiency or resistance. At the same time it causes the hypophosphatemia characteristic of most forms of osteomalacia. The action of parathyroid hormone may be determined by the loss of the permissive action of vitamin D in osteomalacia with a strongly reduced concentration of vitamin D in the blood and the tissues (p. 876). In extreme vitamin-D deficiency, parathyroid hormone no longer acts on the bones (ARNAUD, 1966). In most cases, the

increased parathyroid hormone activity is expressed as the known, specific changes in the skeleton, associated with fibrous osteitis (TORRANI, 1924; BALL, 1960; FISCHER, 1968) together with the changes of osteomalacia.

The pathophysiology of the *renal form of secondary hyperparathyroidism* is more complex. We have already shown that hypocalcemia of long duration is the cause. Renal disorders in existence for years or going back to youth are the main cause of the severe form of renal hyperparathyroidism. These include congenital malformations of the urinary tract with chronic renal insufficiency and urinary tract infection, or chronic pyelonephritis. As in osteomalacia, the hypocalcemia is either inadequately or almost completely corrected by the increased release of parathyroid hormone. The elevated phosphate level resulting from glomerular insufficiency remains uncorrected – there is no raised phosphate excretion since the parathyroid hormone succeeds in acting only inadequately on the diminished number of renal tubules. The effects of secondary hyperparathyroidism on the bone tissue are identical to those of primary hyperparathyroidism. They are, however, complicated by osteomalacia, which is usually quite severe, although it varies widely from case to case, and which is seen together with the changes brought about by fibro-osteoclastic osteitis (MACH, FOLLIS, BERNER, and BALL). Osteoid is occasionally seen in primary hyperparathyroidism (p. 917), whereas this is always present in renal secondary hyperparathyroidism. The osteomalacia of chronic renal insufficiency is probably due to suppression of the hydroxylation of 25-hydroxycholecalciferol into 1,25-dihydroxycholecalciferol by the kidney, leading to a state which resembles nutritional vitamin-D deficiency.

Intestinal absorption of calcium and phosphorus is greatly reduced even in the early course of chronic renal insufficiency (STANBURY). The osteomalacia of chronic renal disorders is identical to the changes seen in nutritional vitamin-D deficiency. It can thus be assumed that the chronic hypocalcemia of the chronic renal failure is due in the first place to calcium malabsorption. In addition, extremely low amounts of calcium and phosphates are usually excreted in the urine, and there is an increase in the serum phosphatase depending on the severity of the secondary hyperparathyroidism. The insufficient vitamin-D activity results in deficient calcification of the newly formed bone matrix with an accumulation of large uncalcified osteoid masses.

In conclusion, the bone changes accompanying the renal manifestation of secondary hyperparathyroidism are a mixed form with fibrosis and osteomalacia. The osteomalacic components of the renal osteopathy can be therapeutically influenced by high doses of vitamin D (p. 938), although there is considerable danger of vitamin-D intoxication and soft-tissue calcification.

Parathyroid hormone secretion is highly stimulated in some cases of renal secondary hyperparathyroidism, which can cause the original hypocalcemia to give way to normocalcemia and finally to hypercalcemia. This increase of the serum calcium due to the mobilization of calcium from the skeleton is accompanied by a similar rise in serum phosphates, also due to an increase in bone resorption. The phosphate cannot be removed by the damaged renal tubules. In such cases, extensive fibrous osteitis with bone pains develops, with radiologically severe subperiosteal resorption. In addition to this, when the Ca × P product exceeds 70, metastatic calcification can occur. This represents hyperfunction of the parathyroids which has become apparently autonomous. This hypothetical assumption has recently been supported by new evidence obtained from the findings with renal transplants. It has been shown that after successful renal transplantations, the pre-operative hypocalcemia not infrequently reverts to an often temporary hypercalcemia with values of 11–13.5 mg/100 ml, with the clinical picture of the skeletal hyperparathyroidism with calcification of the soft tissues and associated with the danger of renal damage due to nephrocalcinosis (MCPHAUL and MCINTOSH). In some cases, spontaneous correction of the hypercalcemia has occurred after several weeks or months, indicating the regression of the parathyroid hyperfunction. In other cases, however, hypercalcemia has lasted months or years and subtotal parathyroidectomy has had to be performed, thus curing the clinical and radiological symptoms of the fibrous osteitis and normalizing the serum calcium concentration (MCPHAUL, p. 938). The hypothesis that fully autonomous (tertiary) hyperparathyroidism can develop from secondary hyperparathyroidism is questionable, since all forms of primary and secondary hyperparathyroidism can be suppressed with calcium infusions (POTTS, 1971; BINSWANGER, 1972). Subtotal parathyroidectomy is discussed on p. 938 and tertiary hyperparathyroidism on p. 939.

6. Clinical Features and X-Ray Findings

The bone changes in secondary hyperparathyroidism are rarely clinically evident, mainly in cases of chronic uremia. Depending on

the pathogenesis fibroosteoclasia or osteomalacia is predominant (cf. section on the pathologic histology of primary hyperparathyroidism, p. 916). Pains in the extremities are the most frequent symptom, associated with deformations in adolescents (genu valgum and varum, pectus carinatum with rachitic rosary, kyphoscoliosis, epiphysiolysis or necrosis of the femoral head). The growth in children is retarded (renal dwarfism, renal rickets). The extensive osteopathy occasionally leads to severe bone pains with difficulty in walking, and exceptionally to invalidity. In severe osteomalacic osteopathy, tetany and muscular weakness are occasionally observed in the regions of the deltoids, the supraspinatus, and the iliopsoas, and can be associated with the characteristic waddling gait. Vitamin D often brings about an impressive correction of this osteomalacic myopathy (STANBURY). A painful form of pruritus is occasionally associated with secondary hyperparathyroidism of renal insufficiency.

The radiological findings present a combination of the changes of osteitis fibrosa cystica generalisata and/or of osteomalacia. The osteitis fibrosa is visible as subperiosteal resorption and cannot be differentiated from that occurring in primary hyperparathyroidism (p. 913). The acro-osteolysis and the ground-glass appearance of the skull are also identical to the findings in primary hyperparathyroidism (p. 915). Even bone cysts and epulides have been observed in rare cases of renal osteitis. Focal osteosclerosis alone is more frequently encountered in secondary hyperparathyroidism than in primary hyperparathyroidism (p. 916). The banded sclerosis of the vertebral bodies is considered especially characteristic. It is a ribbon-like thickening of the spongiosa along the upper and lower plates of the lumbar vertebrae. Each sclerotic band thus partly borders a clear intervertebral disc and a clear central part of the vertebral body, thus producing zebra-like transverse stripes (rugger-jersey spine). Where the osteomalacia predominates, typical Looser's zones can be found in all the possible localizations of classic osteomalacia. Bending of the bones also occurs in some cases, e.g. heart-shaped deformation of the pelvis. Changes in the epiphyseal regions have been observed in young subjects and in children. These changes are similar to those found in nutritional rickets. In some cases, acro-osteolysis of the metaphyseal end of the diaphysis can develop in addition to rachitic metaphysis, so that the diaphyseal ends appear to have a moth-eaten, perforated structure. In rare cases where the Ca × P product is above 70, X-rays reveal periarticular, subcutaneous, and arterial calcifications and nephrocalcinosis.

7. Differential Diagnosis

Although primary hyperparathyroidism differs from secondary hyperparathyroidism in the early stages, differentiation between the two conditions can be difficult in advanced cases. Primary hyperparathyroidism with nephrocalcinosis, uremia and skeletal involvement is often clinically, biochemically and even pathoanatomically similar to advanced secondary hyperparathyroidism due to chronic renal insufficiency. The histological findings in the parathyroids in secondary hyperparathyroidism (microadenomatous hyperplasia, p. 934) are indistinguishable from those in primary hyperparathyroidism due to primary haperplasia of the chief cells (p. 908). As has already been mentioned, the histological skeletal findings are identical in both conditions.

A few clinical characteristics which can be used for differentiations in individual cases are outlined here. A history of nephrolithiasis is very common in primary hyperparathyroidism, whereas it is hardly ever found in secondary hyperparathyroidism. Chronic renal insufficiency, when present, is almost always connected with severe nephrocalcinosis in primary hyperparathyroidism; nephrocalcinosis rarely arises in secondary hyperparathyroidism resulting from chronic uremic nephropathy. If the symptoms date back to youth, the secondary hyperparathyroidism is usually associated with rachitic deformations. This is not seen in primary hyperparathyroidism since this disorder is rare in children. Pruritus, subcutaneous and periarticular calcifications, osteosclerosis (rugger-jersey spine), and highly elevated immunoreactive parathyroid hormone levels (depending on the antibody used) are rarely seen in primary hyperparathyroidism, but are present quite often in secondary hyperparathyroidism.

8. Therapy

The therapy of secondary hyperparathyroidism in chronic uremia is restricted to cases with clinical skeletal symptoms, tetany, myopathy or metastatic tissue calcifications. However, the cause of the secondary hyperparathyroidism, chronic uremia, has to be treated first, including the nephrogenic anemia.

Before treatment with vitamin D can be started the serum phosphorus must be lowered or brought back into the normal range with oral aluminium hydroxide (Amphojel). The purpose is to reduce the calcium × phosphate

product to less than 70 and if possible to avoid soft tissue calcifications. Only then the patients receive vitamin D orally, in an initial daily dose of 0.25–0.5 mg (1 mg = 40000 IU). If no beneficial effect is seen after several weeks, the dose is gradually increased to 12.5 mg daily. The therapeutic success can be recognized from the normalization of the serum calcium concentration, the disappearance of skeletal pains, improvement of the general condition and normalization of the radiological findings. However, since vitamin D acts slowly and accumulates in tissues such as the liver, the dose must be increased cautiously. In order to avoid any risk of vitamin-D overdosage it is essential to check the serum levels of calcium, phosphorus and phosphatase regularly. An increase of the serum calcium above the normal range should be avoided, as should high serum phosphate levels, which result in a Ca × P product of over 70.

The fall of the alkaline phosphate to normal values can be considered a sign of an established cure. When there is the slightest suspicion of vitamin-D overdosage, the treatment must be interrupted for several weeks and the clinical course observed. Because of the accumulation of vitamin D in tissues, interruption of the treatment is perfectly permissible as a precaution even if there is no special suspicion of overdosage. With the onset of recovery, the vitamin-D therapy can be discontinued while the patient remains under clinical observation, or maintenance therapy with 0.25 mg daily or 3 times weekly is introduced.

In hyperphosphatemia the serum phosphate must first be lowered with aluminium hydroxide (see above). This is even more important if the serum calcium is normal or increased and the serum phosphorus is also high, thus causing an elevated Ca × P product. Here, vitamin D would also have a favorable effect on the osteopathy, but this cure must be purchased at the price of hypercalcemia with soft tissue calcification, nephrocalcinosis and progressive renal failure. If the condition is incurable, subtotal or total parathyroidectomy may be indicated in such cases. Conditions necessary for this procedure are relatively good general health and normal or not highly elevated blood pressure. In severe renal insufficiency renal dialysis must be started first. After subtotal parathyroidectomy in which several grams of parathyroid tissue can be removed and according to WILSON a residue of 200–300 mg or 7/8 of the total glandular tissue is left behind, the levels of the serum calcium and phosphorus fall alarmingly. Vitamin D in relatively high doses of 2.5–6.25 mg per day can now be administered. The osteopathy heals clinically and radiologically, the metastatic calcifications may disappear, and the general condition improves considerably. Any doubts about the justification of subtotal parathyroidectomy in cases of chronic uremic renal disorders with a favorable prognosis have been removed by convencing reports of successful cures with considerable improvement in the skeletal pains and the general condition.

When patients in chronic renal failure are dialyzed, the calcium concentration in the dialysis bath is critical for the maintenance of secondary hyperparathyroidism. FOURNIER (1971) has demonstrated that the immunoreactive parathyroid hormone concentrations can be lowered if a calcium concentration of 8 mg/100 ml rather than 5–6 mg/100 ml is used. However, the serum phosphate must first be lowered and kept in a normal range in order to avoid soft tissue calcifications. This is achieved by oral administration of aluminium hydroxide (Amphojel). Thus a highly successful conservative treatment of severe secondary hyperparathyroidism is possible and subtotal parathyroidectomy is avoidable in a certain number of patients on chronic dialysis.

9. Normocalcemic Secondary Hyperparathyroidism

JAN A. FISCHER

The increased secretion of parathyroid hormone in the presence of hypocalcemia has already been described on p. 872 as a cause of secondary hyperparathyroidism.

When patients with steatorrhea and hypocalcemia are treated with vitamin D for short periods, the serum calcium becomes normal within days. Hyperparathyroidism persists although the stimulus for the fall in serum calcium is absent. This can be supported by the rise in the phosphate excretion index, the hydroxyproline excretion in the urine and by the increased number of osteoclasts in bone biopsies. One patient remained normocalcemic for at least 9 months before dying of encephalomalacia. The post mortem revealed generalized fibroosteoclasia and hyperplasia of the parathyroid glands. Another patient showed clinical signs of hyperparathyroidism after having been normocalcemic for over 18 months.

The phosphaturia cannot be due to vitamin D since the doses used do not produce a pathologic increase of the urinary phosphate excretion even in patients who have undergone parathyroidectomy. When the parathyroids are intact the phosphate excretion is usually reduced due to suppression of the parathyroid

glands by the simultaneous rise in the serum calcium concentration (ALBRIGHT, 1948). In contrast to the action of parathyroid hormone, vitamin D reduces hydroxyproline excretion (KLEIN, 1963).

FISCHER (1968) has described this condition as normocalcemic secondary hyperparathyroidism. It is presumably a special form of secondary hyperparathyroidism with an altered feedback mechanism in the parathyroids. In spite of the normalized serum calcium concentration, the intestinal malabsorption persists with a reduction in calcium absorption. Lowering of the serum magnesium concentration as a possible additional cause of the increased release of parathyroid hormone in spite of the normocalcemia cannot always be excluded.

The differentiation from tertiary apparently autonomous hyperparathyroidism is not always easy. The homeostatic regulation of the calcium concentration in the serum is intact in the presence of a normal serum calcium concentration.

The normocalcemic form of secondary hyperparathyroidism is also seen in nutritional rickets of infancy. As a rule the phosphate concentration in the serum is diminished and immunoreactive parathyroid hormone concentrations increased (ARNAUD, 1972). It is possible that vitamin-D deficiency independent of the normal serum calcium concentration is the cause of normocalcemic secondary hyperparathyroidism (FISCHER, 1973).

In summary, this is a form of hyperparathyroidism in which hypocalcemia, the usual stimulus for an increased release of parathyroid hormone, is absent and in which no hypercalcemia is seen, suggesting that factors other than the serum calcium concentration stimulate parathyroid hormone secretion.

I. Tertiary Hyperparathyroidism

M. WERNLY

This condition has already been discussed on p. 904 in the etiology of primary hyperparathyroidism, where it was mentioned that a parathyroid adenoma can sometimes develop from secondary hyperparathyroidism, giving rise to the clinical picture of primary hyperparathyroidism. This condition was given the name tertiary hyperparathyroidism by ST. GOAR in 1963, and DAVIS in 1956 was the first to remove a parathyroid adenoma from a patient with malabsorption. It must be added that this condition cannot be sharply differentiated from the clinical picture of severe secondary hyperparathyroidism, which STANBURY and others treat with subtotal parathyroidectomy (p. 938). In both conditions autonomous ("primary") hyperparathyroidism develops from reactive (secondary) hyperparathyroidism.

Table 7. Diagnosis of normocalcemic secondary hyperparathyroidism. CaS = serum calcium; PEI = phosphate excretion index (immunoreactive parathyroid hormone increased)

Hyperparathyroidism	CaS	OH-proline in urine	Fibro-osteoclasts	PEI
Primary	↑(→)			
Secondary	↓	↑(→)	↑(→)	↑(→)
Secondary normocalcemic	→			
Tertiary	↑			

In some cases all four parathyroids are hyperplastic, reflecting secondary hyperparathyroidism. There are, however, confirmed cases of tertiary hyperparathyroidism due to a solitary adenoma after kidney transplantation. Tertiary hyperparathyroidism can only be differentiated clinically from secondary hyperparathyroidism, and not by the histological findings in the parathyroids. There are transitional stages between normocalcemic secondary hyperparathyroidism and tertiary hyperparathyroidism. They are characterized by hypercalcemia, a rise in the phosphate excretion index, and increased hydroxyproline excretion in the urine. An increase in the number of osteoclasts is almost always demonstrable. The parathyroids secrete parathyroid hormone seemingly autonomously, independent of any regulation by the serum calcium, and it can therefore only be differentiated from primary hyperparathyroidism by a previous history of secondary hyperparathyroidism. It often arises after renal transplantation (HERDMAN and MCINTOSH).

In most patients the serum calcium concentration becomes normal after some weeks or months. When it remains elevated subtotal parathyroidectomy is necessary.

The suppressibility of the immunoreactive parathyroid hormone concentrations by calcium infusions in primary hyperparathyroidism and in secondary hyperparathyroidism of renal insufficiency (p. 911) makes it appear likely that tertiary hyperparathyroidism is not autonomous either. However, the few patients with secondary renal hyperparathyroidism who develop hypercalcemia after renal transplantation have not been fully investigated.

K. Laboratory Investigations and Function Tests in Disorders of the Parathyroids

B. COURVOISIER

BERSON and YALLOW introduced a radioimmunoassay for estimation of the parathyroid hormone concentration in plasma or serum (p. 870). Since then the sensitivity and the precision of the measurement of the immunoreactive parathyroid hormone concentration in the serum (IPTH) has been considerably improved (REISS, 1968; POTTS, 1969; ARNAUD, 1971). The principles of radioimmunological estimations of hormones have been discussed elsewhere (estimation of insulin, p. 814). The reader is referred to the literature for other methods of investigation which are of particular importance for research purposes. These include balance investigations, quantitative morphometric evaluation of bone biopsies after labeling with tetracycline, and dynamic investigations of bone metabolism with trace substances, such as stable strontium, radioactive calcium (^{45}Ca, ^{47}Ca) and radioactive strontium (^{85}Sr). They have opened up new aspects in research into bone metabolism. Only clinically applicable methods in current use are discussed below (p. 873). It must be emphasized that measurement of the immunoreactive parathyroid hormone concentration in the serum (IPTH) is essential in the diagnosis of all doubtful cases of hyperparathyroidism. The detailed clinical evaluation of IPTH is discussed on p. 897.

1. Diagnosis of Primary Hyperparathyroidism

a) Hypercalcemia

The normal range for serum calcium is 9.0–10.5 mg/100 ml. Values vary according to the methods used and from one laboratory to the other, so that it is essential that the normal range is known in each laboratory.

Hypercalcemia is a very important biological finding in the diagnosis of primary and tertiary hyperparathyroidism (several recent reports in the literature have, however, emphasized the occurrence of normocalcemia in primary hyperparathroidism (p. 904)). The serum calcium must be estimated by a very accurate chemical method. The levels of the serum proteins must be taken into account in the interpretation of the results obtained (the serum calcium is decreased in the presence of a low blood level of protein). The acid-base balance must also be considered (ionized serum calcium is raised in acidosis and reduced in alkalosis). Prolonged venous constriction must be avoided when the blood sample is taken since the calcium can rise due to hemoconcentration (DENT, 1962). Apart from this, cork stoppers must not be used to seal containers with blood samples because they contain calcium.

Ultrafiltration of plasma and estimation of the free diffusable (ionized and complex-bound) calcium are necessary for calcium clearance investigations. In doubtful cases the estimation of the ionized calcium concentration is indicated.

b) Hypophosphatemia

The normal range for serum phosphate is 4.0–6.0 mg/100 ml in children and 2.5–3.5 mg 100 ml in adults. Like hypercalcemia, hypophosphatemia is a mandatory symptom of primary hyperparathyroidism. Its value, however, is limited by the fact that phosphate is not adequately excreted in renal insufficiency which is often found in primary hyperparathyroidism. In these patients the serum phosphate concentration can be normal or increased. In the presence of normal renal function, hypophosphatemia is frequently seen in primary hyperparathyroidism.

The *combination of hypercalcemia and hypophosphatemia* used to be considered almost pathognomonic for the diagnosis of hyperparathyroidism; it has now been shown, however, that in hypercalcemia of other etiologies, serum phosphate is usually normal or slightly elevated, but that this is not a constant finding, since hypercalcemia can also be combined with hypophosphatemia in some circumstances, e.g. metastatic skeletal carcinomatosis and hypercalcemia following carcinoma without skeletal metastases. Since the levels of parathyroid hormone and an immunoreactive substance similar to parathyroid hormone in the blood have been found to be elevated in several cases of tumor hypercalcemia (p. 927), the above combinations appear understandable, at least in these cases (RIGGS, 1971).

c) Alkaline Phosphatase

The level of this enzyme in the serum appears to be an index of osteoblastic activity. Numerous methods of measurement are reported, whose results are given in different units. The normal ranges are generally well defined for each method. An increase in alkaline phosphatase is a common but not mandatory finding in primary hyperparathyroidism. Elevated levels are found almost exclusively in cases with

demonstrable skeletal involvement. The alkaline phosphatase may also be elevated in numerous other conditions with skeletal involvement, e.g. Paget's disease, osteomalacia, rickets, renal osteopathy, osteogenic sarcoma, metastatic skeletal carcinomatosis, etc. Elevated values of alkaline phosphatase can only be interpreted as the result of a skeletal disorder if hepatobiliary disorders have been excluded. See above, p. 856 for information on special forms of alkaline phosphatase.

Acid serum phosphatase can be elevated in primary hyperparathyroidism (GRUNDIG, 1965).

d) Hypercalciuria

Estimation of the urinary excretion of calcium in 24 hours is important in the diagnosis of primary hyperparathyroidism. Since this investigation is thought to reveal the endogenous calcium turnover, i.e. the endogenous calcium loss, it should be carried out while the patient is receiving a calcium-deficient diet.

The patient receives a calcium-deficient diet containing about 150 mg calcium/day for at least 4 days. The 24-hour calcium excretion is measured on the last 2 or 3 days. Hydroxyproline can also be measured in the same urine samples providing the diet is deficient in collagen as well (Table 8). As urinary calcium is influenced by the quantity of sodium ingested in the diet and excreted by the kidney (the renal tubular handling of calcium is similar to that of sodium), the sodium content in the diet and urine must be taken into account if the clinical significance of urinary calcium is to be appreciated. In cases where strict dietary restrictions are impossible, as for example in outpatients, a low-calcium diet can be limited to the omission of milk and cheese (butter permitted) and a collagen-deficient diet limited to the omission of meat and gelatine. The 24-hour urine should be collected in a clean container and bacterial infection prevented by the addition of a few crystals of thymol. Before a sample is removed for analysis, the urine should be acidified and well stirred (to dissolve precipitates containing phosphates and oxalates).

Interpretation: With a calcium-deficient diet, not more than 200 mg (strict diet) or 250 mg (diet avoiding milk and cheese) calcium should be excreted in the urine in 24 hours. Hypercalciuria ranging between 200 and 600 mg is found in primary hyperparathyroidism, depending on the degree of hypercalcemia. Hypercalciuria is not a constant finding, however, because parathyroid hormone increases the tubular reabsorption of calcium. Hypercalciuria is more frequently seen in vitamin-D intoxication, or in hypercalcemia not caused by parathyroid hormone, where parathyroid hormone secretion is suppressed.

Table 8. Quantitative low-calcium and low-hydroxyproline diet

1600 calories 160 mg calcium 600 mg phosphorus	40 g proteins 2 g collagen 280 mg hydroxyproline
Breakfast:	
Tea or coffee	
Bread	50 g
Butter	10 g
Sugar	20 g
Jam	50 g
Lunch:	
Beef or veal	100 g
Artichoke hearts	50 g
or Canned asparagus	110 g
or Grated carrots	50 g
or Canned French beans	50 g
or Tomatoes	200 g
Potatoes	150 g
Oil	20 g
Bread	25 g
or Bananas	100 g
or Canned peach compôte	150 g
or Apples	150 g
or Canned pears	75 g
Dinner:	
Egg (not to be replaced by meat)	1
Noodles	40 g
Butter	10 g
Salad	25 g
Oil, salt, pepper, vinegar	10 g
Same fruits as lunch	
Salt, pepper. Sugar: ad libitum	
Drinks:	
Distilled water, no other mineral water, juice of one lemon per day, tea, black coffee, tea made with distilled water	

e) Estimation of Hydroxyproline

Measurement of the urinary excretion of hydroxyproline permits an approximate quantitative assessment of bone turnover and especially of bone resorption (C, 3, b, p. 863). Hydroxyproline is an end product of the metabolic breakdown of collagen. About 50% of it arises from the skeleton.

Interpretation: The mean 24-hour urinary excretion of hydroxyproline is 30 mg in healthy subjects (range 10–40 mg). During growth, hydroxyproline excretion is higher (50–90 mg/24 hours). Abnormally high values are found in numerous conditions with skeletal involvement, as well as in primary hyperparathyroidism with marked skeletal involvement.

f) Renal Phosphate Excretion Tests

These tests are based on the fact that parathyroid hormone increases the renal phosphate excretion by inhibition of the tubular reabsorption. Except for the estimation of the phosphate clearance, which does not require the measurement of the creatinine clearance, the method is identical, but the calculations for obtaining the various indices discussed below are not.

Method. The phosphate intake must be adequate for several days immediately before the test. The patient therefore receives a normal diet containing 700 to 1 400 mg phosphorus/24 hours. The test starts at 8 a.m. in a fasting patient, and lasts four hours. The patient empties his bladder just prior to the test, and collects the urine 4 hours later. A blood specimen is taken in the middle of the test. The phosphate and creatinine levels are determined in both serum and urine. The phosphate excretion can be expressed in various ways.

Phosphate Clearance.

$$C = \frac{\text{P urine} \times V}{\text{P serum}}$$

V = volume of urine

The normal phosphate clearance is 10.8 ml ± 2.7 ml/min. It usually exceeds 15 ml/min in primary hyperparathyroidism. The measurement of the phosphate clearance allows only poor discrimination between normal and hyperparathyroid subjects and it also varies widely with the phosphate intake.

Tubular Reabsorption of Phosphate (TRP). The creatinine clearance corresponds approximately to the glomerular filtration rate. The ratio between phosphate and creatinine clearances thus indicates the proportion of filtered glomerular phosphate not reabsorbed by the tubules. The ratio is calculated as follows:

$$\frac{C_p}{C_{cr}} = \frac{\text{P urine} \times \text{creatinine serum}}{\text{P serum} \times \text{creatinine urine}}$$

C_p = phosphate clearance
C_{cr} = creatinine clearance

Usually these results are expressed as the proportion of the glomerular filtrate reabsorbed by the tubules: *Tubular reabsorption of phosphate (TRP)*

$$TRP = \left[\left(1 - \frac{C_p}{C_{cr}}\right) \times 100\right] \%$$

$$= \left[\left(1 - \frac{\text{P urine} \times \text{creatinine serum}}{\text{P serum} \times \text{creatinine urine}}\right) \times 100\right] \%$$

Interpretation: The normal range for TRP is 85–95%. In primary hyperparathyroidism it is frequently lowered although there is significant overlap between the two groups.

Phosphate Excretion Index (PEI). NORDIN and FRASER (1965) include the serum phosphate level in their calculations to take into account the phosphate load of the kidney filtration. The PEI is calculated from the following formula:

$$PEI = \frac{C_p}{C_{cr}} - 0.055 \times \text{P serum} + 0.07 .$$

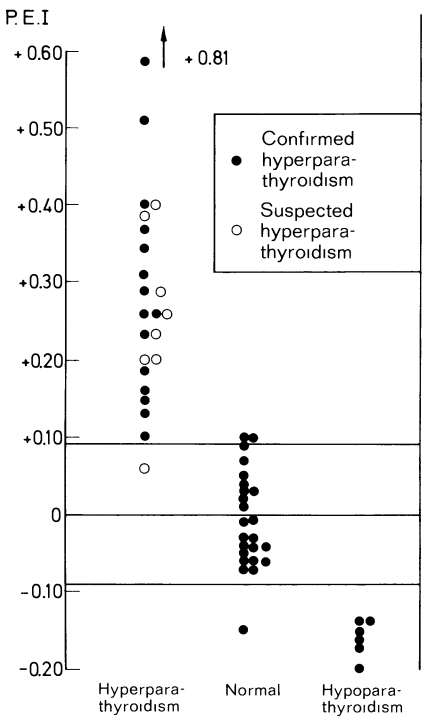


Fig. 34. Phosphate excretion index in hyperparathyroidism, hypoparathyroidism and in normal subjects (NORDIN, 1965)

The normal range is −0.09 to +0.09. Regardless of the serum phosphate level, a PEI lower than −0.09 or higher than +0.09 indicates an abnormal urinary phosphate excretion. In most of their cases of primary hyperparathyroidism, NORDIN and FRASER (1965) found the PEI to be above +0.09. However, overlaps do occur with normal subjects with the PEI and the TRP.

Index of Phosphorus Excretion (IPE). This is a modified phosphate excretion index (PEI), which appears to allow better discrimination between hyperparathyroid and normal subjects (NORDIN, 1968).

The IPE is calculated from the following formula:

$$IPE = \frac{P\ urine \times creatinine\ serum}{creatinine\ urine} - \frac{P\ serum - 2.5}{2}$$

The normal range is -0.5 to $+0.5$.

Maximal Tubular Reabsorption of Phosphate (Tm/GFR). The Tm/GFR can either be measured directly with the infusion of phosphate, or calculated and read off from the nomogram of BIJOVET (1969) (Fig. 35).

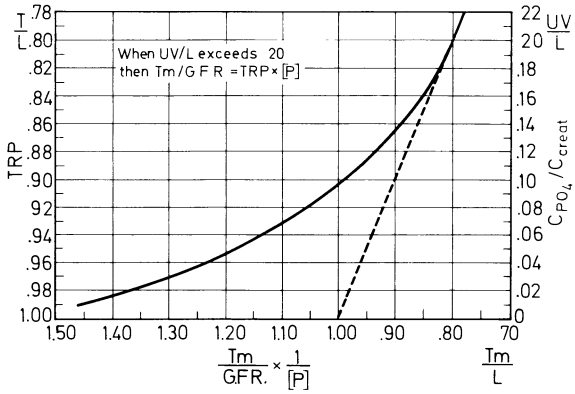


Fig. 35. Nomogram for the estimation of the $\frac{Tm}{GFR}\ \frac{18}{p}$ from the TRP or the C_{PO_4}/C_{Creat}. (After MORGAN, 1972)

The TRP and the C_p/C_{cr} have to be calculated, and the $\frac{Tm}{GFR} \times \frac{1}{P\ serum}$ read off from the nomogram of BIJOVET. This value has then to be multiplied by the serum phosphate concentration and the result is the *Tm/GFR*.

The normal range of the Tm/GFR is 22–42 mg/liter. It is lower in hyperparathyroidism and higher in hypoparathyroidism. The discrimination between normal and hyperparathyroid subjects appears to be better than with any other renal phosphate excretion test used.

g) Parathyroid Inhibition by Induced Hypercalcemia

Parathyroid activity is controlled by the concentration of calcium ions in the plasma. In healthy subjects, induced hypercalcemia thus causes temporary inhibition or suppression of parathyroid activity which becomes apparent as a sudden fall in the phosphate clearance. It used to be thought that the parathyroids acted autonomically and their activity could not be suppressed in primary hyperparathyroidism. If this were so, exogenous hypercalcemia would be followed by only a very slight change in phosphate clearance or none at all. Since sensitive methods for the determination of parathyroid hormone have been available, it has been demonstrated repeatedly that in hyperparathyroidism the parathyroids or their adenomas are not autonomous and their secretion is influenced by the concentration of the calcium ions in the blood (POTTS, 1972; BINSWANGER, 1973), so that both the following tests have lost their theoretical basis. However, in hyperthyroidism the influence of a further rise in calcium seems to produce a less pronounced fall in the phosphate clearance, and the tests might have some empirical value if no better criteria are available.

KYLE *and* HAAS *Method.* Phosphate and creatinine clearances are measured on the first day of the test between 8 and 10 a.m. Blood is taken at 9. An intravenous drip containing 15 mg Ca^{++}/kg body weight in 500 ml physiological saline is set up on the evening of the first day (10 ml 20% calcium gluconate = 180 mg Ca^{++}). The infusion is started at 9 p.m. and stopped at midnight. Blood is taken before and after the insfusion. On the second day, phosphate and creatinine clearances are measured under exactly the same conditions as on the previous day. This procedure of spreading out the test over 2 days is thought to eliminate a false interpretation due to variations of phosphaturia at different times of day (p. 854). This test is only of value when the serum calcium rises by at least 3 mg during the infusion.

Interpretation: Normal phosphate clearance is 10.8 ± 2.7 ml. Following hypercalcemia, the phosphate clearance can fall by more than 50% in healthy subjects. This fall is less than 25% in some patients with primary hyperparathyroidism.

Pak's Method. It has been shown that during a calcium infusion the change in the renal phosphate excretion is not dependent only upon the parathyroid inhibition. The calcitonin secretion is stimulated by the hypercalcemia and acts directly on the kidney to increase phosphate excretion. Calcium infusion also acts directly on the phosphate clearance independently of the PTH and calcitonin levels.

The stimulated endogenous calcitonin and the direct hypercalcemic action oppose the parathyroid inhibition. False-positive values are thus obtained in normal subjects by this test. PAK *et al.* have noticed that these two mecha-

nisms are active only for the first 12 hours after a four-hour calcium infusion. The inhibition of parathyroid hormone lasts 24 hours or longer. If phosphate clearance is determined over 12 hours (12th to 24th hour) after the calcium infusion, the source of error can be eliminated and the parathyroid response to hypercalcemia tested.

The patient is given a constant diet for three days; the daily intake of calcium, phosphorus and sodium is approximately 400 mg, 1000 mg, and 100 mEq respectively. The first day on the diet is the equilibrium day; the second is the control day for calcium infusion. At 9 a.m. on the third day of the diet the infusion of calcium is begun: 15 mg calcium (as calcium gluconate)/kg of body weight diluted to 500 ml with normal saline and administered intravenously over four hours. Amounts of sodium and fluid equivalent to those in the infusion are removed from the diet on the day of the infusion to keep the intake constant. Food, medication and fluid are given at the same time each day.

Urine is collected every 12 hours for two days beginning at 9 a.m. of the control day. Phosphorus and creatinine levels are measured in each specimen. The urinary phosphorus level is expressed as the ratio (by weight) to urinary creatinine level (mg phosphorus/mg creatinine).

The urinary excretion of phosphorus during the second half of the day of the calcium infusion is compared with that of the corresponding time period of the control day.

In normal patients, the ratio P/C_2 is decreased between -29% and -80% (mean value -52.7%); in hyperparathyroid patients this ratio varies between -25% and $+26\%$ (mean -7.7%).

h) Changes in the Reabsorption of Phosphate after Administration of Parathyroid Hormone (Becker)

(This test should be avoided if parathyroid hormone determinations are available, because of parathyroid antibody formation.) An injection of parathyroid hormone administed to healthy subjects causes a fall in the index of tubular reabsorption of phosphate. This fall is much more striking in the presence of hypoparathyroidism than in the healthy individual (Courvoisier). There is, however, a slight resistence to exogenous parathyroid hormone in cases with an elevated endogenous parathyroid hormone secretion.

Method: After supper, the subject to be tested receives nothing more to eat or drink until the following morning. The bladder is emptied at 8 p.m. and the urine discarded. A slow intravenous infusion of 1000 ml of 5% glucose is set up immediately afterwards. All urine is collected during the subsequent 12 hours, and phosphorus and creatinine measured. The infusion is regulated to last until between 7 and 8 a.m. At 8 a.m. blood is taken for the estimation of phosphorus and creatine. The patient is then discharged and instructed to pursue normal activities. At 8 p.m. on the second day, a similar infusion is given, this time, however, with the addition of 200 USP units of Lilly parathyroid extract (1 ml = 100 USP U = 20 U after Collip). The parathyroid extract must always be tested in a healthy subject (same method). An intracutaneous test should be performed on the person beforehand as a precaution to prevent allergic reactions to the parathyroid extract. (An intracutaneous injection of the extract diluted to 10 times its volume with physiological saline is given.) The index of tubular reabsorption (% TRP) is calculated on both days of the test.

Interpretation: The injection of parathyroid hormone causes a significant fall in tubular reabsorption of phosphate in healthy subjects and in hypercalcemia not due to primary hyperparathyroidism (11.5–27.6% fall in TRP). In primary hyperparathyroidism the reabsorption falls by a maximum of 6.8%.

i) Cortisone Test after Dent *for the Differential Diagnosis of Hypercalcemia*

This test is used by Dent for the differentiation of hypercalcemia due to primary hyperparathyroidism from hypercalcemia of other etiologies. Shulman was the first to notice that hypercalcemia in sarcoidosis can be corrected by glucocorticoids. Later, the same observation was made in vitamin-D intoxication. Glucocorticoids inhibit bone resorption and the release of calcium into the serum.

Method: 150 mg cortisone or 30 mg prednisone is given daily for 10 days. The serum calcium is determined before the test and on the 5th, 8th and 10th days.

Interpretation: From Dent's work, it can be concluded that cortisone and its derivatives do not affect hypercalcemia associated with primary hyperparathyroidism, whereas they cause a fall in serum calcium in hypercalcemia of other etiologies. Other authors, however, have occasionally demonstrated exceptions in both directions (Thomas and Gwinup). In spite of this, the cortisone test can be recommended

for the differential diagnosis of hypercalcemia because of its simplicity.

2. Diagnosis and Differential Diagnosis of Hypoparathyroidism

a) Calcemia and Phosphatemia

(Hypocalcemia; Hyperphosphatemia)

Measurements used in the diagnosis of primary hyperparathyroidism are also used for this purpose (p. 943).

b) Alkaline Phosphatase

This parameter is normal.

c) Urinary Calcium Excretion

Hypoparathyroidism is characterized by a very low urinary excretion of calcium. The calcium excretion depends to a large extent on the serum calcium concentration. The urinary calcium excretion allows no clear-cut discrimination between hypoparathyroid and normal subjects, one reason for this being that the tubular calcium reabsorption is inhibited in the absence of parathyroid hormone. It appears that in normocalcemic control subjects on a calcium-deficient diet the 24-hour urinary calcium excretion always exceeds 50 mg.

d) Phosphate Excretion Measurement

In contrast to hyperparathyroid patients, only a poor discrimination between normal and hypoparathyroid subjects is possible, whatever phosphate excretion test is used.

e) Ellsworth-Howard Test

The phosphaturic effect of parathyroid hormone or extract is used to differentiate pseudohypoparathyroidism from other forms of parathyroid insufficiency.

Method: On the first day of the test, hourly samples of urine are collected for 6 hours. Volume, phosphate and creatinine are measured in each sample. This investigation should be carried out at the time of day when phosphaturia is most stable, i.e. during the afternoon (after lunch). On the second day, 6 samples of urine are collected at the same time intervals, and 200 USP U Lilly parathyroid hormone (1 ml = 100 USP U) are injected at the end of the third hour. If possible, the same test should be performed at the same time in a healthy subject, using the same brand of parathyroid extract with the same production number to examine its phosphaturic potency. To prevent allergic reactions to the parathyroid extract, an intracutaneous test is performed beforehand with a solution of the extract diluted to 10 times its volume with physiological saline; 0.1 ml is used for the intracutaneous injection.

Interpretation: In healthy subjects, there is a 100% increase in the excretion of phosphate in the first hour after the injection of the parathyroid extract. This rise is even more pronounced in postoperative hypoparathyroidism and in certain cases of idiopathic hypoparathyroidism. Pseudohypoparathyroidism, however, is thought to be characterized by the fact that there is only a slight response to parathyroid extract or none at all.

f) Parathyroid Stimulation Tests

These tests should allow diagnosis of undected, latent, or partial hypoparathyroidism. Investigations of this type have been performed in thyroidectomized subjects with a normal serum calcium concentration. The method is described below, although it is of more theoretical than practical interest.

g) Artificial Hypocalcemia with EDTA (JONES *and* FOURMAN)

Ethylene-diamine-tetra-acetate (EDTA) chelates calcium. This chelate has a high affinity for calcium and makes it unavailable to the system. Thus, this salt causes hypocalcemia.

Method: Na_3-EDTA is given by intravenous infusion: 70 mg/kg body weight in 500 ml 5% glucose. The infusion is given over 2 hours (20 ml 2% procaine is added to prevent or alleviate pain which may be caused by the infusion). Blood is taken before and 2, 4, 8, 12, and 24 hours after the start of the infusion. Serum calcium not bound to EDTA is measured by titration.

Interpretation: In healthy subjects, the level of calcium not bound to EDTA falls by 2–3 mg/100 ml towards the end of the infusion (after 2 hours) and reaches normal levels again after 12 hours. It is assumed that this rapid correction of the hypocalcemia is due to mobilization of calcium caused by stimulation of the parathyroid hormone secretion. In parathyroid insufficiency, the fall is often more pronounced after 2 hours than in healthy subjects. The slow return to normal is particularly striking. It is 12 hours, or sometimes more than 24 hours, before normal levels are restored. This is thought to be due to an insufficiency of the parathyroid hormone secretion.

References

Reviews

POTTS, J.T., JR., DEFTOS, L.: Parathyroid hormone, thyrocalcitonin, vitamin D, bone and bone mineral metabolism. In Duncan's diseases of metabolism, 6th ed. Philadelphia: Saunders 1969.

RASMUSSEN, H.: The parathyroids. In: WILLIAMS, R.H. (ed.), Textbook of endocrinology, 4th ed. Philadelphia: Saunders 1968.

History

For earlier literature see: WERNLY, M. In: Labhart, Klinik der inneren Sekretion, p. 939. Berlin-Göttingen-Heidelberg: Springer 1957.

BERSON, S.A., YALOW, R.S.: Clinical applications of plasma parathyroid hormone. Amer. J. Med. **50**, 623 (1971).

COPE, O.: The story of hyperparathyroidism at the Massachusetts General Hospital. New Engl. J. Med. **274**, 1174 (1966).

COPP, D.H., CAMERON, E.C., CHENEY, B.A., DAVIDSON, A.G.F., HENZE, K.G.: Evidence for calcitonin — A new hormone from the parathyroid that lowers blood calcium. Endocrinology **70**, 638 (1962).

— CHENEY, B.A.: Calcitonin — a hormone from the parathyroid which lowers the calcium level of the blood. Nature (Lond.) **193**, 381 (1962).

HIRSCH, P., GAUTHIER, F., MUNSON, P.L.: Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as a factor affecting the response to parathyroidectomy in rats. Endocrinology **73**, 244 (1963).

TASHJIAN, A.H.: Homeostasis of plasma calcium: effects of actinomycin D, parathyroidectomy and thyrocalcitonin. Endocrinology **77**, 375 (1965).

TENENHOUSE, A., ARNAUD, C.D., ANAST, C., RASMUSSEN, H.: Thyrocalcitonin: its nature and mechanism of action. Proceedings of the VIth Pan-American Congr. of Endocrinology. Excerpta med. Found. **112**, 172 (1965).

Anatomy and Histology

For earlier literature see: WERNLY, M. In: Labhart, Klinik der inneren Sekretion, p. 939. Berlin-Göttingen-Heidelberg: Springer 1957.

ERDHEIM, J.: Normale und pathologische Histologie der Thyreoidea, Parathyreoidea und Hypophyse. Beitr. path. Anat. **33**, 158 (1903).

— Über Schilddrüsenaplasie, Geschwülste des Ductus thyreoglossus. Über einige menschliche Kiemenderivate. Beitr. path. Anat. **35**, 366 (1904).

— Tetania parathyreopriva. Mitt. Grenzgeb. Med. Chir. **16**, 632 (1906).

— Über Epithelkörperchenbefunde bei Osteomalacie. Sitzungsber. d. kaiserl. Akad. d. Wissensch. Wien, naturw. Klasse, **116**, Abt. III, Heft 6, S. 311 (1907).

— Rachitis und Epithelkörperchen. Denkschr. K.K. Akad. Wissensch., naturw. Klasse 1914, S. 90.

GEIS, N.P.: The parathyroid glands. Ann. Surg. **47**, 523 (1908).

HALSTED, W.S., EVANS, H.M.: The parathyroid glandules. Their blood supply, and their preservation in operation upon the thyroid gland. Ann. Surg. **46**, 489 (1907).

HAMPERL, H.: Onkocyten und Onkocytome. Virchows Arch. path. Anat. **335**, 452 (1962).

HOLZMANN, K., LANGE, R.: Zur Cytologie der Glandula parathyreoidea des Menschen. Weitere Untersuchungen an Epithelkörperadenomen. Z. Zellforsch. **58**, 759 (1963).

LANGE, R.: Zur Histologie und Cytologie der Glandula parathyreoidea des Menschen. Licht- und elektronenmikroskopische Untersuchungen an Epithelkörperadenomen. Z. Zellforsch. **53**, 765 (1961).

MUNGER, B.L., ROTH, S.I.: The cytology of the normal parathyroid gland of man and Virginia deer. A Light an electromicroscopic study with morphologic evidence of secretory activity. J. Cell Biol. **16**, 379 (1963).

ROTH, S.I.: Pathology of the parathyroids in hyperparathyroidism. Arch. Path. **73**, 495 (1962).

— Recent advances in parathyroid gland pathology. Amer. J. Med. **50**, 612 (1971).

— MUNGER, B.L.: The cytology of the adenomatous, atrophic parathyroid glands of man. Virchows Arch. path. Anat. **335**, 389 (1962).

SHELDON, H.: On the water-clear cell in the human parathyroid gland. J. Ultrastruct. Res. **10**, 377 (1964).

WERNLY, M., BERDJIS-CHAMSI, CH.: Les parathyroides humaines. Basel: Benno Schwabe & Co. 1946.

Biochemistry and Physiology of Calcium and Phosphate Metabolism

Reviews

ALBRIGHT, F., REIFENSTEIN, E.C., JR.: The parathyroid glands and metabolic bone disease. Baltimore: Williams & Wilkins 1948.

ARNAUD, C.D., TENENHOUSE, A.M., RASMUSSEN, H.: Parathyroid hormone. Ann. Rev. Physiol. **29**, 349 (1967).

AURBACH, G.D., POTTS, J.T., JR.: Parathyroid hormone. Amer. J. Med. **42**, 1 (1967).

CONNOR, C.L., BRONNER, F. (eds.): Mineral metabolism, an advanced treatise. New York: Academic Press 1964.

COURVOISIER, B., BERTHOUD, E., ZAHND, G., GRANDJEAN, A.: Données actuelles sur la physiologie et la pathologie clinique des parathyroides. Helv. med. Acta **26**, Suppl. 38, No. 5 (1959).

Editorial: Action of parathyroid hormone. New Engl. J. Med. **269**, 924 (1963).

FISCHER, J.A.: Die Wirkungsweise des Parathormons. Schw. med. Wschr. **96**, 273 and 321 (1966).

FLEISCH, H., BLACKWOOD, H.J.J., OWEN, M. (eds.): Calcified tissue 1965. Berlin-Heidelberg-New York: Springer 1966.

FOSTER, G.V.: Calcitonin (Thyrocalcitonin). New Engl. J. Med. **279**, 349 (1968).

FOURMAN, P., ROYER, P.: Calcium metabolism and the bone, 2nd ed. Oxford: Blackwell 1968.

FROST, H.M. (ed.): Bone biodynamics. Boston: Little, Brown 1964.

GAILLARD, P.J., TALMAGE, R.V., BUDY, A.M. (eds.): The parathyroid glands. Chicago: University of Chicago Press 1965.

GOLDHABER, P.: Some current concepts of bone physiology. New Engl. J. Med. **266**, 870 (1962).

GREEP, R.O., TALMAGE, R.V. (eds.): The parathyroids. Springfield: Thomas 1961.

HARRIS, W.H., HEANEY, R.P.: Skeletal renewal and metabolic bone disease. New Engl. J. Med. **280**, 193, 247, 303 (1969).

HIRSCH, P.F., MUNSON, P.L.: Thyrocalcitonin. Physiol. Rev. **49**, 548 (1969).

LICHTWITZ, A., PARLIER, R.: Calcium et maladies métaboliques de l'os (3 vol.). Par: L'expansion éd. 1964.

NEUMAN, W.F., NEUMAN, M.W.: The chemical dynamics of bone mineral. Chicago: University of Chicago Press 1958.

PROCKOP, D.J., KIVIRIKKO, K.I.: Relationship of hydroxyproline excretion to collagen metabolism. Ann. intern. Med. **66**, 1243 (1967).

RASMUSSEN, H.: Parathyroid hormone, nature and mechanism of action. Amer. J. Med. **30**, 112 (1961).

— On the mechanism of action of the parathyroid hormone. In: KARLSON, P. (ed.), Mechanisms of hormone action, p. 131. Stuttgart: Thieme, G. 1965.

— (ed.): Parathyroid hormone, thyrocalcitonin and related drugs. In: International encyclopedia of pharmacology and therapeutics, sect. 51, vol. I. Oxford: Pergamon Press 1970.
TALMAGE, R.V., BÉLANGER, L.F. (eds.): Parathyroid hormone and thyrocalcitonin (Calcitonin). Amsterdam: Excerpta Medica 1968.
— MUNSON, P.L. (eds.): Calcium, parathyroid hormone and the calcitonins. Amsterdam: Excerpta Medica 1972.
TAYLOR, S. (ed.): Calcitonin 1967: Symposium on thyrocalcitonin and the C cells. London: Heinemann 1968.
— (ed.): Calcitonin 1969: Proceedings of the 2nd international symposium. London: Heinemann 1970.
TENENHOUSE, A., RASMUSSEN, H., HAWKER, C.D., ARNAUD, C.D.: Thyrocalcitonin. Ann. Rev. Pharmacol. **8**, 319 (1968).
VAES, G.: La résorption osseuse et l'hormone parathyroidienne. Bruxelles: Visscher 1967.

Calcium Metabolism

ALCOCK, N., MACINTYRE, I.: Inter-relation of calcium and magnesium absorption. Clin. Sci. **22**, 185 (1962).
AVIOLI, L.V., MCDONALD, J.E., SINGER, R.A., HENNEMAN, P.H.: A new oral isotopic test of calcium absorption. J. clin. Invest. **44**, 128 (1965).
AXELROD, D.R.: Sodium chloride induced calciuria in hyperparathyroidism. J. clin. Endocr. **26**, 207 (1966).
BINSWANGER, U.: Nierenfunktion unter peroraler Calciumtherapie. Schweiz. med. Wschr. **97**, 140 (1967).
BIRGE, S.J., PECK, W.A., BERMAN, M., WHEDON, G.D.: Study of calcium absorption in man. J. clin. Invest. **48**, 1705 (1969).
CAMERON, J.R., SORENSON, J.: Measurement of bone mineral in vivo. Science **142**, 230 (1963).
CLARK, I.: Effect of magnesium ions on calcium and phosphorus metabolism. Amer. J. Physiol. **214**, 348 (1968).
— BÉLANGER, L.: The effects of dietary magnesium on calcium, phosphate and skeletal metabolism. Calc. Tiss. Res. **1**, 204 (1967).
COBURN, J.W., MASSRY, S.G., CHAPMAN, L.W., KLEEMAN, C.R.: Effects of sodium or calcium infusions on renal magnesium excretion with normal and reduced filtered load. Clin. Res. **15**, 354 (1967).
CUSHARD, W.G., JR., CREDITOR, M.A., CANTERBURY, J.M., REISS, E.: Parathyroid function during pregnancy. 53rd Meeting Endocrine Society, A-64 (1971).
DANOWSKI, T.S.: Clinical endocrinology, vol. III. Calcium, phosphorus, parathyroids and bone. Baltimore: Williams & Wilkins 1962.
DE GRAZIA, J.A., JVANOVICH, P., FELLOWS, H., RICH, C.: A double isotope method for the measurement of intestinal absorption of calcium in man. J. Lab. clin. Med. **66**, 822 (1965).
DENT, C.E.: Some problems of hyperparathyroidism. Brit. med. J. **1962II**, 1419.
DUARTE, C.G., WATSON, J.F.: Calcium reabsorption in proximal tubule of the dog nephron. Amer. J. Physiol. **212**, 1355 (1967).
EDWARDS, N.A., HODGKINSON, A.: Studies of renal functions in patients with idiopathic hypercalciuria. Clin. Sci. **29**, 327 (1965).
EPSTEIN, F.H., BECK, D., CARONE, F.A., LEVITIN, H., MANITIUS, A.: Changes in renal concentrating ability produced by parathyroid extract. J. clin. Invest. **38**, 1214 (1959).
FLEISCH, H., RUSSEL, R.G.G., BISAZ, S., CASEY, P.A., MÜHLBAUER, R.C.: The influence of pyrophosphate analogues (diphosphonates) on the precipitation and dissolution of calcium phosphate in vitro and in vivo. Calc. Tiss. Res. **2**, Suppl. 10 (1968).
GERSHBERG, H., HECHT, A., ZENEIDA, J.: Growth hormone and blood calcium homeostasis. J. clin. Endocr. **27**, 1492 (1967).
HARGREAVES, T., ROSE, G.A.: The reproducibility of the balance method in man as applied to calcium and phosphorus. Clin. Sci. **28**, 573 (1965).
HARRISON, D.G., LONG, C.: The calcium content of human erythrocytes. J. Physiol. (Lond.) **199**, 367 (1968).
HOWARD, J.E.: Reflections on calcium habits in man. Canad. med. Ass. J. **99**, 41 (1968).
HYTTEN, F.E., LEITCH, I.: The physiology of human pregnancy. Oxford: Blackwell 1964.
ISAKSSON, B., LINDHOLM, B., SJÖGREN, B.: 11. Dermal calcium losses. Metabolism **16**, 303 (1967b).
— SJÖGREN, B.: A critical evaluation of the calcium balance technic. 1. Variation in fecal output. Metabolism **16**, 295 (1967a).
LAAKE, H.: The action of corticosteroids on the renal reabsorption of calcium. Acta endocr. (Kbh.) **34**, 60 (1960).
LASSITER, W.E., FRICK, A., RUMRICH, G., ULLRICH, K.S.: Influence of ionic calcium on the water permeability of proximal and distal tubules in the rat kidney. Pflügers Arch. ges. Physiol. **285**, 90 (1965).
LAZOR, M.Z., ROSENBERG, L., CARBONE, P.: Studies of calcium metabolism in multiple myeloma with ^{47}calcium and metabolic balance techniques. J. clin. Invest. **42**, 1238 (1963).
LEMANN, J., JR., PIERING, W.F., LENNON, E.J.: Possible role of carbohydrate induced calciuria in calcium oxalate kidney stone formation. New Engl. J. Med. **280**, 232 (1969).
LUTWAK, L.: Absorption of calcium in man. Calcified tissues, 1965, p. 198. Berlin-Heidelberg-New York: Springer 1965.
MACMANUS, J., HEATON, F.W.: The effect of magnesium deficiency on calcium homeostasis in the rat. Clin. Sci. **36**, 297 (1969).
MASSRY, S.G., COBURN, J.W., CHAPMAN, L.W., KLEEMAN, C.R.: The effect of long-term desoxycorticosterone acetate administration on the renal excretion of calcium and magnesium. J. Lab. clin. Med. **71**, 212 (1968).
MAZZUOLI, G.F., TERRENATO, L., COEN, G.: Calcium homeostasis in hypothyroidism and hypoparathyroidism evaluated by means of calcium infusion. 4th European Symposium on calcified tissues, p. 71. Amsterdam: Excerpta Medica 1966.
MOORE, E.W.: Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. J. clin. Invest. **49**, 318 (1970).
NORDIN, B.E.C.: Measurement and meaning of calcium absorption. Gastroenterology **54**, 294 (1968).
SODE, J., SABOL, J., SEROM, E., CANARY, J.: Correlative study of ^{47}Ca absorption in man. J. clin. Invest. **47**, 92a (1968).
SUKI, W.N., EKNOYAN, G., RECTOR, F.C., JR., SELDIN, D.W.: The renal dilating and concentrating mechanism in hypercalcemia. Nephron **6**, 50 (1969).
— SCHWETTMANN, R.S., RECTOR, F.C., JR., SELDIN, D.W.: Effect of chronic mineralocorticoid administration on calcium excretion in the rat. Amer. J. Physiol. **215**, 71 (1968).
THIERS, R.E., VALLEE, B.L.: Distribution of metals in subcellular fractions of rat liver. J. biol. Chem. **226**, 911 (1957).
VELLAR, O.D., ASKEVOLD, R.: Studies on sweat loss of nutrients. Scand. J. clin. Lab. Invest. **22**, 65 (1968).
WALSER, M.: Ion association. VI. Interactions between calcium, magnesium, inorganic phosphate, citrate and protein in normal human plasma. J. clin. Invest. **40**, 723 (1961).
WHO: Technical Report, p. 230 (1962).
YENDT, E.R., GAGNÉ, R.J.A., COHANIM, M.: The effect of thiazides in idiopathic hypercalciuria. Amer. J. med. Sci. **251**, 449 (1966).

Phosphate Metabolism

ARNAUD, C. D., TSAO, H. S., LITTLEDIKE, T.: Radioimmunoassay of human parathyroid hormone in serum. J. clin. Invest. **50**, 21 (1971).

AVIOLI, L. V., MCDONALD, J. E., HENNEMAN, P. H., LEE, S. W.: The relationship of parathyroid activity to pyrophosphate excretion. J. clin. Invest. **45**, 1093 (1966).

BIJOVET, O. L. M., MORGAN, D. B., FOURMAN, P.: The assessment of phosphate reabsorption. Clin. chim. Acta **26**, 15 (1969).

BRODEHL, J., GELLISSEN, K.: Der Einfluß des Angiotensin II auf die tubuläre Phosphatrückresorption beim Menschen. Klin. Wschr. **44**, 1171 (1966).

CORVILAIN, J., ABRAMOW, M.: Effect of growth hormone on tubular transport of phosphate in normal and parathyroidectomised dogs. J. clin. Invest. **43**, 1608 (1964).

ELLISON, E., STRAUMFJORD, J. V., HUMMEL, J. P.: Buffer capacities of human blood and plasma. Clin. Chem. **4**, 452 (1958).

FALLS, W. F., JR., CARTER, N. W., RECTOR, F. C., JR., SELDIN, D. W.: The mechanism of impaired phosphate reabsorption in chronic renal failure. Clin. Res. **14**, 74 (1966).

FAIRHURST, B. J.: Urinary phosphate excretion in hypoparathyroidism. Lancet **1963 I**, 302.

FISCHER, J. A., BINSWANGER, U., Blum, J. W.: The acute parathyroid hormone response to changes in ionized calcium during phosphate infusions in the cow. Europ. J. clin. Invest. **3**, 151 (1973).

GOLDSMITH, R. S., SIEMSON, A. W., MASON, A. D., JR., FORTLAND, M.: Primary role of plasma hydrocortisone concentration in the regulation of the normal forenoon pattern of urinary phosphate excretion. J. clin. Endocr. **25**, 1649 (1965).

ISAKSSON, B., AHLSSON, L.: A critical evaluation of the calcium balance technic. III. The theoretical phosphorus balance. Metabolism **16**, 314 (1967).

LOTZ, M., ZISMAN, E., BARTTER, F. C.: Evidence for a phosphorus-depletion syndrome in man. New Engl. J. Med. **278**, 409 (1968).

RIEDLER, G. F., SCHEITLIN, W. A.: Hypophosphataemia in septicemia: Higher incidence in gram-negative than in gram-positive infections. Brit. med. J. **1969/1**, 753.

STANBURRY, S. W.: Some aspects of disordered renal tubular function. Adv. intern. Med. **9**, 231 (1958).

SUKI, W. N., MARTINEZ-MALDONADO, M., ROUSE, D., TERRY, A.: Effect of expansion of extracellular fluid volume on renal phosphate handling. J. clin. Invest. **48**, 1888 (1969).

TRANSBOL, I., HALVER, B.: Relation of renal glycosuria and parathyroid function in hypercalcemic sardoidosis. J. clin. Endocr. **27**, 1193 (1967).

WALSER, M.: Ion association. VI. Interactions between calcium, magnesium, inorganic phosphate, citrate and protein in normal human plasma. J. clin. Invest. **40**, 723 (1961).

WEBSTER, G. D., MANN, J. B., HILLS, A. G.: The effect of phosphate infusion upon renal phosphate clearance in man: Evidence for tubular secretion. Metabolism **16**, 797 (1967).

Bone Metabolism

ADAMSON, L. F., INGBAR, S. H.: Selective alteration by triiodothyronine of amino acid transport in embryonic bone. Endocrinology **81**, 1362 (1967).

ARNOLD, J. S.: Dynamics of metabolic bone disease, eds. PEARSON, O. H., JOPLIN, G. F. Oxford 1964.

— JEE, W. S. S.: Bone growth and osteoclastic activity of plutonium. Amer. J. Anat. **101**, 367 (1957).

AUBERT, J. P., MILHAUD, G.: Méthode de mesure des principales voies du métabolisme calcique chez l'homme. Biochim. biophys. Acta (Amst.) **39**, 122 (1960).

BARTTER, F. C.: Hypophosphatasia. The metabolic basis of inherited disease, 2nd ed., STANBURY, J. B., WYNGARDEN, J. B., FREDRICKSON, D. S., eds. New York: McGraw Hill 1966.

BAUD, C. A.: Structure et fonctions des ostéocytes dans les conditions normales et sous l'influence de l'extrait parathyroidien. Schweiz. med. Wschr. **98**, 717 (1968).

BAUER, G. C., CARLSSON, A., LINDQUIST, B.: Metabolism and homeostasic function of bone. Mineral metabolism, vol. I B, p. 609, COMAR, C., BRUNNER, F., eds. New York: Academic Press 1961.

BÉLANGER, L. F.: Osteocytic osteolysis. Calc. Tiss. Res. **4**, 1 (1969).

BELL, G. H., DUNBAR, O., BECK, J. S., GIBB, A.: Variations in strength of vertebrae with age and their relation to osteoporosis. Calc. Tiss. Res. **1**, 75 (1967).

BERNSTEIN, D. S., SADOWSKI, N., HEGSTED, D. M., GURI, C. D., STORE, F. J.: Prevalence of osteoporosis in high- and low-fluoride areas in North Dakota. J. Amer. med. Ass. **198**, 499 (1966).

BINSWANGER, U., FISCHER, J. A., MERZ, W., SCHENK, R., MAYER, G., UEHLINGER, E.: Thyrocalcitonin und primärer Hyperparathyreoidismus. Analyse knochenbioptischer Befunde. 14. Symposium Endokrinologie (1968a).

BRONNER, F., AUBERT, J. P.: Bone metabolism and regulation of the blood calcium level in rats. Amer. J. Physiol. **209**, 887 (1965).

BURCKHARDT, R.: Technische Verbesserungen und Anwendungsbereich der Histobiopsie von Knochenmark und Knochen. Klin. Wschr. **44**, 326 (1966).

CARNIERO, J., LEBLOND, C. P.: Role of osteoblasts and odentoblasts in secreting collagen of bone and dentin, as shown by radioautography in mice given tritium labeled glycerine. Exp. Cell Res. **18**, 291 (1959).

CARTIER, P.: La minéralisation du cartilage ossifiable. VIII. Les pyrophosphates du tissu osseux. Bull. Soc. Chim. biol. (Paris) **41**, 573 (1959).

EISENBERG, E., GORDAN, G. S.: Skeletal dynamics in man measured by nonrodioactive strontium. J. clin. Invest. **40**, 1809 (1961).

FLANAGAN, B., NICHOLS, E., JR.: Metabolic studies of human bone in vitro. II. Changes in hyperparathyroidism. J. clin. Invest. **44**, 1795 (1965).

FLEISCH, H., MAERKI, J., RUSSELL, R. G. G.: Effect of pyrophosphate on dissolution of hydroxyapatite and its possible importance in calcium homeostasis. Proc. Soc. exp. Biol. (N. Y.) **122**, 317 (1966a).

— NEUMANN, W. F.: Mechanisms of calcification: role of collagen, polyphosphates and phosphatase. Amer. J. Physiol. **200**, 1296 (1961).

— RUSSELL, R. G. G.: Pyrophosphate, polyphosphates and calcium homeostasis. In: Encyclopedia of pharmacology (H. RASMUSSEN, ed.). New York: Pergamon Press 1970.

— — STRAUMANN, F.: Effect of pyrophosphate on hydroxyopatite and its implications in calcium homeostasis. Nature (Lond.) **212**, 901 (1966b).

FRASER, R., HARRISON, M., IBBERTSON, K.: The rate of calcium turnover in bone. Quart. J. Med., N.S. **29**, 85 (1960).

FROST, H. M.: Bone remodelling dynamics. Springfield: Ch. C. Thomas 1963.

GAILLARD, P. J.: Parathyroid and bone in tissue culture. In: The parathyroids (GREEP, R. O., TALMAGE, R. V., eds.). Springfield: Ch. C. Thomas 1961.

GARN, S. M., ROHMANN, G. G., WAGNER, B.: Bone loss as a general phenomenon in man. Fed. Proc. **26**, 1729 (1967).

GLIMCHER, M. J., CRANE, S. M.: The nature of inorganic phosphate binding. In: Radioisotope and bone (LACROIX, P., BUDY, A. M., eds.). Oxford: Blackwell 1962.

GONZALES, F., KARNOVSKY, M. J.: Electron microscopy of osteoclasts in healing fractures of rat bone. J. biophys. biochem. Cytol. **9**, 299 (1961).

HANCOX, N. M., BOOTHROYD, B.: Structure-function relationship in osteoclasts. In: Mechanisms of hard tissue

destruction (SOGNNAES, R.F., ed.). Washington: Amer. Ass. Adv. Sci. 1963.
HEANEY, R.P., BAUER, G.C.H., BRONNER, F., DYMLING, J.F., LAFFERTY, F.W., NORDIN, B.E.C., RICH, C.: A normal reference standard for radiocalcium turnover and excretion in humans. J. Lab. clin. Med. **64**, 21 (1964).
— WHEDON, G.D.: Radiocalcium studies of bone formation rate in human metabolic bone disease. J. clin. Endocr. **18**, 1246 (1958).
JOHNSTON, C.C., JR., SMITH, D.M., YU, P.-A., DEISS, W.P., JR.: In vivo measurements of bone mass in radius. Metabolism **17**, 1140 (1968).
JOWSEY, J.: Quantitative microradiography. Amer. J. Med. **40**, 485 (1966).
KLEIN, L., LAFFERTY, F.W., PEARSON, O.H., CURTISS, P.H., JR.: Correlation of urinary hydroxyproline, serum alkaline phosphatase and skeletal calcium turnover. Metabolism **13**, 272 (1964).
KÖLLIKER, A.: Die normale Resorption des Knochengewebes und ihre Bedeutung für die Entstehung der typischen Knochenformen. Leipzig: Vogel 1873.
KROKOWSKI, E.: Möglichkeiten zur Bestimmung des Skelett-Calciumgehaltes in der Klinik. Dtsch. med. Wschr. **91**, 60 (1966).
LEE, W.R., MARSHALL, J.H., SISSONS, H.A.: Calcium accretion and bone formation in dogs. An experimental comparison between the results of ^{45}calcium kinetic analysis and tertacycline labeling. J. Bone Jt. Surg. B **47**, 157 (1965).
LEMANN, J. JR., LITZOW, J.R., LENNON, E.J.: The effects of chronic acid loads in normal man: Further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. J. clin. Invest. **45**, 1608 (1966).
MARSHALL, J.H., ROWLAND, R.E., JOWSEY, J.: Microscopic metabolism of calcium in bone. V. The paradox of diffuse activity and long term exchange. Radiat. Res. **10**, 258 (1959).
MCDONALD, J.E., MAMOLO, N., HENNEMAN, P.H.: Urinary hydroxyproline as an index of bone resorption. Clin. Res. **13**, 329 (1965).
MERZ, W.A., SCHENK, R.K.: A quantitative histological study on bone formation in human cancellous bone. Acta anat. (Basel), in press (1970a).
— — Quantitative structural analysis of human cancellous bone. Acta anat. (Basel), in press (1970b).
MÜLLER, J., SCHENK, R.: Knochenstruktur und Knochenumbau. Med. Mitt. (Melsungen) **40**, 45 (1966).
NEUMAN, W.F.: The milieu intérieur of bone: CLAUDE BERNARD revisited. Fed. Proc. **28**, 1846 (1969).
— NEUMAN, M.W.: The chemical dynamics of bone mineral. Chicago: University of Chicago Press 1958.
PAK, C.Y.C., BARTTER, F.C.: Ionic interaction with bone mineral. Evidence for an isoionic calcium exchange with hydroxypatite. Biochim. biophys. Acta (Amst.) **141**, 401 (1967).
PEACHEY, L.D.: Electron microscopic observations on the accumulation of divalent cations in intramitochondrial granula. J. Cell Biol. **20**, 95 (1964).
POSEN, S.: Alkaline phosphatase. Ann. intern. Med. **67**, 183 (1967).
POSNER, A.S.: Relationship between diet and bone mineral ultrastructure. Fed. Proc. **26**, 1717 (1967).
— Cristal chemistry of bone mineral. Physiol. Rev. **49**, 760 (1969).
PROCKOP, D.J., KIVIRIKKO, K.J.: Relationship of hydroxyproline excretion in urine to collagen metabolism. Ann. intern. Med. **66**, 1243 (1967).
REIDENBERG, M.M., HAAG, B.L., CHANNICK, B.J., SHUMAN, C.R., WILSON, T.G.G.: The response of bone to metabolic acidosis in man. Metabolism **15**, 236 (1966).
RIGGS, B.L., BASSINGTHWAIGHT, J.B., JOWSEY, J.: ^{45}Calcium distribution in dog bone determined by a new method of autoradiographic analysis by computer. Clin. Res. **15**, 430 (1967).
ROSENBAUM, J.L., RAMIREZ, O., SAMUELS, S., BREST, A.N., MOYER, J.H.: Effect of sudden blood calcium depletion on phosphorus excretion. Endocrinology **74**, 266 (1964).
ROWLAND, R.E.: Radioisotopes in the skeleton: Late observations in the distribution of radium in the human skeleton. Minneapolis: Univ. of Minnessota Press 1960.
RUSSELL, R.G.G., CASEY, P., BISAZ, S., FLEISCH, H.: Further studies on the role of pyrophosphate in mineralized tissues and on the function of phosphatases. Proc. 5th Europ. Symp. Calc. Tiss., Bordeaux (1968).
SCHENK, R.K., MERZ, W.A., MUELLER, J.: A quantitative histological study on bone resorption in human cancellous bone. Acta anat. (Basel) **74**, 44 (1969b).
— Figures (1969).
SCHIBLER, D., RUSSELL, R.G.G., FLEISCH, H.: The inhibition by condensed phosphates of aortic calcification induced by vitamin D_3 in rats. Clin. Sci. **35**, 363 (1968).
THOMPSON, R.C., JR., GAULL, G.E., HORWITZ, S.J., SCHENK, R.K.: Hereditary hyperphosphatasia. Amer. J. Med. **47**, 209 (1969).
TROTTER, M., BROMAN, G.E., PETERSON, R.R.: Densities of bones of white and negro skeletons. J. Bone Jt Surg. A **42**, 50 (1960).
VAES, G.: La résorption osseuse et l'hormone parathyroidienne. Bruxelles: Visscher 1967.
YOUNG, R.W.: Spezialisation of bone cells. In: Bone biodynamics (FROST, H.M., ed.). Boston: Little, Brown 1964.

Metabolic Bone Disease

ALBRIGHT, F., REIFENSTEIN, E.C., JR.: The parathyroid glands and metabolic bone disease. Baltimore: Williams & Wilkins 1948.
ARNAUD, C., GLORIEUX, F., SCRIVER, C.: Serum parathyroid hormone in X-linked hypophosphatemia. Science **173**, 845 (1971).
ARNOLD, J.S.: Dynamic studies of metabolic bone disease, PEARSON, O.H., JOPLIN, G.F., eds. Oxford 1964.
BAYLINK, D., WERGEDAHL, J., STAUFFER, M., RICH, C.: Effects of fluoride on bone formation, mineralization, and bone resorption in the rat. In: Fluoride in medicine (VISCHER, T.L., ed.), p. 37. Berne: Huber 1970.
BARRETT, A.J., SLEDGE, C.B., DINGLE, J.T.: Effect of cortisol on synthesis of chondroitinsulfate by embryonic cartilage. Nature (Lond.) **211**, 83 (1966).
BASSETT, C.A.L.: Electro-mechanical factors regulating bone architecture. In: Calcified tissues 1965, p. 78, FLEISCH, H., BLACKWOOD, H.J.J., OWEN, M., eds. Berlin-Heidelberg-New York: Springer 1966.
BAYLINK, D., STAUFFER, M., WERGEDAL, J., RICH, C.: Formation, mineralization, and resorption in vitamin-D deficient rickets. J. clin. Invest. **49**, 1122 (1970).
BELL, N.H.: Dynamics of bone metabolism. Ann. Rev. Med. **18**, 299 (1967a).
— BARTTER, F.C.: Transient reversal of hyperabsorption of calcium and of abnormal sensitivity to vitamin D in a patient with sarcoidosis during episode of nephritis. Ann. intern. Med. **61**, 702 (1964).
— — Studies of ^{47}Ca metabolism in acromegaly. J. clin. Endocr. **27**, 178 (1967b).
BENTZEL, C.J., CARBONE, P.P., ROSENBERG, L.: The effect of prednisone on calcium metabolism and ^{47}Calcium kinetics in patients with multiple myeloma and hypercalcemia. J. clin. Invest. **43**, 2132 (1964).
BERNSTEIN, D.S., SADOWSKY, N., HEGSTED, D.M., GURI, C.D., STORE, F.J.: Prevalence of osteoporosis in high- and low-fluoride areas in North Dakota. J. Amer. med. Ass. **198**, 499 (1968).
BERNBER, E.: Epiphyseal growth zones in oestradiol treated rabbits. Acta endocr. (Kbh.) **57**, 69 (1968).

BINSWANGER, U., SCHENK, R.: Mineralisationsstörung bei chronisch azotämischer Osteodystrophie. Dtsch. med. Wschr. **96**, 1914 (1971).

BRONNER, F., AUBERT, J.P.: Bone metabolism and regulation of the blood calcium levels in rats. Amer. J. Physiol. **209**, 887 (1965).

BURCKHARDT, J.M., JOWSEY, J.: Parathyroid and thyroid hormones in the development of immobilisation osteoporosis. Endocrinology **81**, 1053 (1967).

CLARK, I., GEOFFREY, R.F., BOWERS, W.: Effects of adrenal cortical steroids on calcium metabolism. Endocrinology **64**, 849 (1959).

COLEMAN, R.D., BECKS, H., KOHL, F., VAU, N., COPP, D.H.: Skeletal changes in severe phosphorus deficiency in the rat. Arch. Path. **50**, 209 (1950).

DAUGHDAY, W.H., REEDER, C.: Synchronous activation of DNA synthesis in hypophysectomized rat cartilage by growth hormone. J. Lab. clin. Med. **68**, 357 (1966).

DENT, C.E., FRIEDMAN, M.: Idiopathic juvenile osteoporosis. Quart. J. Med., N.S. **34** 177 (1965).

— SMITH, R.: Nutritional osteomalacia. Quart. J. Med. **38**, 195 (1969).

DOYLE, F., BROWN, J., LACHANCE, C.: Relation between bone mass and muscle weight. Lancet **1970I**, 391.

EDDY, R.L.: Metabolic bone disease after gastrectomy. Amer. J. Med. **50**, 442 (1971).

EISENBERG, E.: Effect of androgens, oestrogens and corticoid on strontium kinetics in man. J. clin. Endocr. **26**, 566 (1966).

FACCINI, J.M.: Inhibition of bone resorption in the rabbit by fluoride. Nature (Lond.) **214**, 1269 (1967).

FISCHER, J.A., BIMSWANGER, U., SCHENK, R.K., MERZ, W.: Histological observations on bone in intestinal malabsorption and vitamin D deficiency. Horm. Metab. Res. **2**, 110 (1970).

FROST, H.M.: Bone remodelling dynamics. Springfield: Ch.C. Thomas 1963.

GARN, S.M., ROHMANN, C.G., WAGNER, B.: Bone loss as a general phenomenon in man. Fed. Proc. **26**, 1729 (1967).

GARNER, A., BALL, J.: Quantitative observations on mineralised and unmineralised bone in chronic renal azotaemia and intestinal malabsorption syndrome. J. Path. Bact. **91**, 545 (1966).

GEDOLIA, I., SCHWARTZ, A., SELA, J., GAZENFIELD, E.: Effects of fluoride on disuse atrophy of bone in rats. Proc. Soc. exp. Biol. (N.Y.) **122**, 657 (1966).

GORDAN, G.S., EISENBERG, E.: The effect of estrogens and corticoid on skeletal kinetics in man. Proc. roy. Soc. Med. **56**, 1027 (1963).

HARTMANN, F.: Die Hydroxyprolinausscheidung im Urin von Normalpersonen und Patienten mit rheumatischen Erkrankungen. Klin. Wschr. **44**, 1053 (1966).

HEANEY, R.P.: A unified concept of osteoporosis. Amer. J. Med. **39**, 877 (1965).

JOWSEY, J., RAISZ, L.G.: Experimental osteoporosis and parathyroid activity. Endocrinology **82**, 384 (1968a).

— SCHENK, R.K., REUTTER, F.W.: Some results of fluoride on bone tissue in osteoporosis. J. clin. Endocr. **28**, 869 (1968b).

— SIMONS, G.W.: Normocalcemia in relation to cortisone secretion. Nature **217**, 1277 (1968c).

KATZ, F.H., KAPPAS, A.: Influence of estradiol and estriol on urinary excretion of hydroxyproline in man. J. Lab. clin. Med. **71**, 65 (1968).

KIMBERG, D.V.: Effects of vitamin D and steroid hormones on the active transport of calcium by the intestine. New Engl. J. Med. **280**, 1396 (1969).

KIVIRIKKO, K.I., LAITINEN, O., LAMBERG, B.A.: Value of urine and serum hydroxyproline in the diagnosis of thyroid disease. J. clin. Endocr. **25**, 1347 (1965).

LAFFERTY, F.W., SPENCER, G.E., JR., PEARSON, O.H.: Effects of androgens, estrogens on bone formation and resorption in osteoporosis. Amer. J. Med. **36**, 514 (1964).

LIBERMANN, U.A., SPERLING, O., ATSMON, A., FRANK, M., MODAN, M., VRIES, A. DE: Metabolic and calcium kinetic studies in idiopathic hypercalciuria. J. clin. Invest. **47**, 2580 (1968).

LITZOW, J.R., LEEMANN, J., JR., LENNON, E.J.: The effect of treatment of acidosis on calcium balance in patients with chronic azotemic renal disease. J. clin. Invest. **46**, 280 (1967).

LOTZ, M., ZISMAN, E., BARTTER, F.C.: Evidence for a phosphorus-depletion syndrome in man. New Engl. J. Med. **278**, 409 (1968).

LUDWIG, G.D., KYLE, G.C., BLANCO, M. DE: "Tertiary" hyperparathyroidism induced by osteomalacia resulting from phosphorus depletion. Amer. J. Med. **43**, 136 (1967).

LUTWAK, L., WHEDON, G.D., LACHANCE, P.A., REID, J.M., LIPSCOMB, H.S.: Mineral, electrolyte and nitrogen balance studies of the Gemini-VII fourteen-day orbital space flight. J. clin. Endocr. **29**, 1140 (1969).

MAUTALEN, C.A.: Circadian rhythm of urinary total and free hydroxyproline excretion and its relation to creatinine excretion. J. Lab. clin. Med. **75**, 11 (1970).

MERZ, W.A., SCHENK, R.K.: A quantitative histological study on bone formation in human cancellous bone. Acta anat. (Basel), in press (1970).

MORGAN, D.B., PATERSON, C.R., WOODS, C.G., PULVERTAFT, C.N., FOURMAN, P.: Search for osteomalacia in 1228 patients after gastrectomy and after operations on the stomach. Lancet **1965II**, 1085.

— PULVERTAFT, C.N., FOURMAV, P.: Effects of age on the loss of bone after gastric surgery. Lancet **1966II**, 772.

MÜLLER, J., SCHENK, R.: Knochenstruktur und Knochenumbau. Med. Mitt. (Melsungen) **40**, 45 (1966).

MUNCK, O.: Osteoporosis and malabsorption of calcium. Successful treatment with large dosis of vitamin D in a 24 year old woman with too little calcified bone without osteoid tissue. Acta orthop. scand. **33**, 407 (1963).

NADARAJAH, A., HARTOG, M., REDFERN, B., THALASSINOS, N., WRIGHT, A.D., JOPLIN, G.F., FRASER, T.R.: Calcium metabolism in acromegaly. Brit. med. J. **1968II**, 797.

NAGENT DE DEUXCHAINES, C., KRANE, S.M.: The treatment of adult phosphate diabetes and Fanconi syndrome with neutral sodium phosphate. Amer. J. Med. **43**, 508 (1967).

PAK, C.Y.C., ZISMAN, E., EVENS, R., JOWSEY, J., DELEA, C.S., BARTTER, F.C.: The treatment of osteoporosis with calcium infusion. Amer. J. Med. **47**, 7 (1969).

PECK, W.A., BRANDT, J., MILLER, I.: Hydrocortisone-induced inhibition of protein synthesis and uridine incorporation in isolated bone cells. Proc. nat. Acad. Sci. (Wash.) **57**, 1599 (1967).

— ZIPKIN, I., WHEDON, G.D.: Fluoride inhibition of bone collagen synthesis. Clin. Res. **13**, 330 (1965).

PHANG, M.J.: Multicompartemental analysis: Calcium metabolism. Ann. intern. Med. **68**, 435 (1968).

POSNER, A.S.: Relationship between diet and bone mineral ultrastructure. Fed. Proc. **26**, 1717 (1967).

RAMSER, J.R., FROST, H.M., FRAME, B., ARNSTEIN, A.R., SMITH, R.: Tetracycline-based studies of bone dynamics in rib of six cases of osteomalacia. Clin. Orthop. **46**, 219 (1966).

RASMUSSEN, H., NAGATA, N., FEINBLATT, J., FAST, D.: Parathyroid hormone, membranes, ions, and encymes. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 299. Amsterdam: Excerpta medica 1968.

RICH, C., ENSINCK, J., IVANOVICH, P.: The effects of sodium fluoride on calcium metabolism of subjects with metabolic bone diseases. J. clin. Invest. **43**, 545 (1964).

RIGGS, B.L., JOWSEY, J., KELLY, P.J., JONES, J.D., MAHER, F.T.: Effect of sex hormones on bone in primary osteoporosis. J. clin. Invest. **48**, 1065 (1969).

ROBICHON, J., GERMAIN, J.P.: Pathogenesis of osteogenesis imperfecta. Canad. med. Ass. J. **20**, 975 (1968).

SCHWARTZ, E., PANARILLO, V.A., SAELI, J.: Radioactive calcium kinetics during high calcium intake in osteoporosis. J. clin. Invest. **44**, 1547 (1965).
SIMMONS, D.J.: Collagen formation and enchondral ossification in estrogen treated mice. Proc. Soc. exp. Biol. (N.Y.) **121**, 1165 (1966).
SLATOPOLSKY, E., CAGLAR, S., PENNELL, J.P., TAGGART, D.D., CANTERBURY, J.M., REISS, E., BRICKER, N.S.: On the pathogenesis of hyperparathyroidism in chronic experimental renal insufficiency. J. clin. Invest. **50**, 492 (1971).
SMITH, D.A., NORDIN, B.E.C.: The relation between calcium balance and hydroxyproline excretion in osteoporosis. Proc. roy. Soc. Med. **57**, 868 (1964).
SPENCER, H., MENCZEL, J., LEWIN, J., SAMACHSON, J.: Absorption of calcium in osteoporosis. Amer. J. Med. **37**, 223 (1964).
STANBURY, S.W., LUMB, G.A.: Parathyroid function in chronic renal failure. Quart. J. Med. **35**, 1 (1966).
STEENBOCK, H., HERTING, D.C.: Vitamin D and growth. J. Nutr. **57**, 449 (1955).
STERN, P.H.: Inhibition by steroids of parathyroid hormone induced Ca^{45} release from embryonic rat bone in vitro. J. Pharmacol. exp. Ther. **168**, 211 (1969).
SZEGNENDERA, J., MADAJEWICS, S.: Calcium metabolism after castration. Lancet **1967II**, 1081.
TOMPSON, G.R., NEALE, G., WATTS, J.M., BOOTH, C.C.: Detection of vitamin D deficiency after partial gastrectomy. Lancet **1966I**, 623.
TROTTER, M., BROMAN, G.E., PETERSON, R.R.: Densities of bones of white and negro skeletons. J. Bone Jt Surg. A **42**, 50 (1960).
VILLANUEVA, A., FROST, H., ILNICKI, L., FRAME, B., SMITH, R., ARNSTEIN, R.: Cortical bone dynamics measured by means of tetracycline labeling in 21 cases of osteoporosis. J. Lab. clin. Med. **68**, 599 (1966).
WHEDON, D.G.: Osteoporosis. In: Clinical endocrinology, vol. 2, p. 349 (ASTWOOD, E.B., CASSIDY, C.E., eds.). New York: Grune & Stratton 1968.
YENDT, E.R., CONNOR, T.B., HOWARD, J.E.: Studies on the mode of action of citrate therapy in rickets. Bull. Johns Hopk. Hosp. **96**, 101 (1955).

Regulation of Calcium and Phosphate Metabolism

Parathyroid Hormone

ANDREATTA, R.H., HARTMANN, A., JÖHL, A., KAMBER, B., MAIER, R., RINIKER, B., RITTEL, W., SIEBER, P.: Synthese der Sequenz 1–34 von menschlichem Parathormon. Helv. chim. Acta **56**, 470 (1973).
ARNAUD, C.D., LITTLEDIKE, T., TSAO, H.S.: Calcium homeostasis and the simultaneous measurement of calcitonin and parathyroid hormone in the pig. In: Calcitonin 1969 (TAYLOR, S., ed.), p. 95. London: W. Heinemann 1970.
— TENENHOUSE, A.M., RASMUSSEN, H.: Parathyroid hormone. Ann. Rev. Physiol. **29**, 349 (1967).
— TSAO, H.S., LITTLEDIKE, D.: Radioimmunoassay of human parathyroid hormone in serum. J. clin. Invest., (1971).
— — OLDHAM, S.B.: Native human parathyroid hormone: an immunochemical investigation. Proc. nat. Acad. Sci. (Wash.) **67**, 415 (1970).
AU, W.Y., RAISZ, L.G.: Restoration of parathyroid responsivness in vitamin-D deficient rats by parenteral calcium or dietary lactose. J. clin. Invest. **46**, 1572 (1967).
AURBACH, G.D.: Isolation of parathyroid hormone after extraction with phenol. J. biol. Chem. **234**, 3179 (1959).
— POTTS, J.T., JR.: Parathyroid hormone. Amer. J. Med. **42**, 1 (1967).
BARNICOT, N.A.: Local action of parathyroid and other tissues on bone in intracerebral grafts. J. Anat. (Lond.) **82**, 233 (1948).
BARRERAS, R.F.: Acid secretion after calcium carbonate in patients with duodenal ulcer. New Engl. J. Med. **282**, 1402 (1970).
— DONALDSON, R.M., JR.: Calcium in gastric hypersecretion, parathyroid adenoma and peptic ulcer. New Engl. J. Med. **276**, 1122 (1967).
BASSETT, C.A.L.: Electro-mechanical factors regulating bone architecture. In: Calcified tissues 1965 (FLEISCH, H., BLACKWOOD, H.J.J., OWEN, M., eds.), p. 78. Berlin-Heidelberg-New York: Springer 1966.
BÉLANGER, L.F., MIGICOWSKY, B.B.: Histochemical evidence of proteolysis in bone: The influence of parathormone. J. Histochem. Cytochem. **11**, 374 (1963).
— RASMUSSEN, H.: Inhibition of osteocytic osteolysis by calcitonin and some anti-growth factors. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 156. Amsterdam: Excerpta Medica 1968.
BERSON, S.A., YALOW, R.S.: Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia and brochogenic carcinoma. Science **154**, 907 (1966).
— — Immunochemical heterogenity of parathyroid hormone in plasma. J. clin. Endocr. **28**, 1037 (1968).
BINSWANGER, U., FISCHER, J.A.: Stimulation of parathyroid hormone (PTH) secretion by intravenous phosphorus infusions in primary hyperparathyroidism. Proc. IX European Symposium on Calcified Tissues, Vienna, in press (1973).
— MERZ, W., SCHENK, R., MAYOR, G., UEHLINGER, E.: Thyrocalcitonin und primärer Hyperparathyreoidismus. Analyse knochenbioptischer Befunde. 14. Symposium Endokrinologie, 1968, p. 95.
BORLE, A.B.: Kinetic analyses of calcium movements in cell cultures. III. Effect of calcium and parathyroid hormone in kidney cells. J. gen. Physiol. **55**, 163 (1970).
BREWER, H.B., JR., FAIRWELL, T., RONAN, R., SIZEMORE, G.W., ARNAUD, C.D.: Human parathyroid hormone: amino-acid sequence of the terminal residues 1–34. Proc. nat. Acad. Sci. (USA) **69**, 3588 (1972).
— RONAN, R.: Bovine parathyroid hormone: amino acid sequence. Proc. nat. Acad. Sci. (Wash.) **67**, 1862 (1970).
BRONNER, F., AUBERT, J.P.: Bone metabolism and regulation of blood calcium levels in rats. Amer. J. Physiol. **209**, 887 (1965).
BUCKLE, R.M., CARE, A.D., COOPER, C.W., GITELMAN, H.J.: The influence of plasma magnesium concentration on parathyroid hormone secretion. J. Endocr. **42**, 529 (1968).
CARE, A.D., SHERWOOD, L.M., POTTS, J.T., JR., AURBACH, G.D.: Perfusion of the isolated parathyroid gland of the goat and sheep. Nature (Lond.) **209**, 55 (1966).
CHASE, L.R., AURBACH, G.D.: Renal adenyl cyclase: Anatomically separate sites for parathyroid hormone and vasopressin. Science **159**, 545 (1968).
— — The effect of parathyroid hormone on the concentration of adenosine 3′,5′-monophosphate in skeletal tissue in vitro. J. biol. Chem. **245**, 1520 (1970).
— MELSON, G.L., AURBACH, G.D.: Pseudohypoparathyroidism: defective excretion of 3′,5′-AMP in response to parathyroid hormone. J. clin. Invest. **48**, 1832 (1969).
COHN, D.V., GRIFFITH, F.D.: The influence of parathyroid extract on oxydative and decarboxylative pathways in bone. In: The parathyroid glands, GAILLARD, P.J., TALMAGE, R.V., BUDY, A.M., eds., p. 231. Chicago: Univ. of Chicago Press 1965.
CUSHARD, W.G., JR., CREDITOR, M.A., CANTERBURY, J.M., REISS, E.: Physiologic hyperparathyroidism in pregnancy. J. clin. Endocr. **34**, 767 (1972).
— SIMON, A.B., CANTERBURY, J.M., REISS, E.: Parathyroid function in sarcoidosis. New Engl. J. Med. **286**, 395 (1972).
DAVIS, R., TALMAGE, R.V.: Evidence for liver inactivation of parathyroid hormone. Endocrinology **66**, 312 (1960).
DOTY, S.B., SCHOFIELD, B.H., ROBINSON, R.A.: The electron-microscopic identification of acid phosphatase in bone cells following parathyroid extract or thyrocalcitonin

administration. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 169. Amsterdam: Excerpta Medica 1968.

— YATES, C.W., LOTZ, W.E., KISECLESK, W., TALMAGE, R.V.: Effect of short term alpha irradiation on parathyroid activity and osteoclast numbers. Proc. Soc. Exp. Biol. (N.Y.) **119**, 77 (1965).

EGAWA, J., NEUMAN, W.F.: Effect of parathyroid extract in the metabolism of radioactive phosphate in kidney. Endocrinology **74**, 90 (1964).

ELIEL, L.P., CHANES, R., HAWRYLKO, J.: Urinary excretion of parathyroid hormone in man. J. clin. Endocr. **25**, 445 (1965).

EPSTEIN, F.H., BECK, D., CARONE, F.A., LEVITIN, H., MANITIUS, A.: Changes in renal concentrating ability produced by parathyroid extract. J. clin. Invest. **38**, 1214 (1959).

FEINBLATT, J., BÉLANGER, L.F., RASMUSSEN, H.: Effect of phosphate infusion on bone metabolism and parathyroid hormone action. Amer. J. Physiol. **218**, 1624 (1970).

FELL, H.B., DINGLE, J.T., WEBB, M.: Studies on the mode of action of excess vitamin A. 4. The specificity of the effect on embryonic chick-limb cartilage in culture and in isolated rat liver lysosomes. Biochem. J. **83**, 63 (1962).

FISCHER, J.A., BINSWANGER, U., BLUM, J.W.: The acute parathyroid hormone response to changes in ionized calcium during phosphate infusions in the cow. Europ. J. clin. Invest. **3**, 151 (1973a).

— — RITTEL, W., DIETRICH, F.M.: Human parathyroid hormone: immunological characterization of antibodies against the synthetic amino-terminal fragment 1–34 and their use in the determination of immunoreactive parathyroid hormone in human sera. In: Endocrinology 1973 (TAYLOR, S., ed.). London: W. Heinemann 1974.

— — SCHENK, R.K., MERZ, W.: Histological observations on bone in intestinal malabsorption and vitamin D deficiency. Horm. Metab. Res. **2**, 110 (1970).

— — — — KISTLER, H.J.: Normocalcemic secondary hyperparathyroidism in malabsorption. Helv. med. Acta **35**, 30 (1969).

— BLUM, J.W., BINSWANGER, U.: The acute parathyroid hormone response to epinephrine in vivo. J. clin. Invest. **51**, in press (1973b).

— OLDHAM, S.B., SIZEMORE, G.W., ARNAUD, C.D.: Calcium-regulated parathyroid hormone peptidase. Proc. mat. Acad. Sci. (USA) **69**, 2341 (1972).

FLANAGAN, B., NICHOLS, E., JR.: Metabolic studies of human bone in vitro: 11. Changes in hyperparathyroidism. J. clin. Invest. **44**, 1795 (1965).

— NICHOLS, G., JR.: Bone matrix turnover and balance in vitro. I. The effects of parathyroid hormone and thyrocalcitonin. J. clin. Invest. **48**, 595 (1969).

FLEISCH, H., MAERKI, J., RUSSELL, R.G.G.: Effect of pyrophosphate on dissolution of hydroxyapatite and its possible importance in calcium homeostasis. Proc. Soc. Exp. Biol. (N.Y.) **122**, 317 (1966).

— RUSSELL, R.G.G.: Pyrophosphate, polyphosphates and calcium homeostasis. In: Encyclopedia of pharmacology (H. RASMUSSEN, ed.). New York: Pergamon Press 1970.

FORLAND, M., STRENDJARD, N.M., PALOYAN, E., COX, A.: Bone density studies in primary hyperparathyroidism. Arch. intern. Med. **122**, 236 (1968).

FOURMAN, P.: Parathyroids and tubular function. Mem. Soc. Endocr. **13**, 133 (1963).

GAILLARD, P.J.: Parathyroid and bone in tissue culture. In: The parathyroids (GREEP, R.O., TALMAGE, R.V., eds.). Springfield: Ch.C. Thomas 1961.

— Protein metabolism and the effect of parathyroid hormone on bone. Tex. Rep. Biol. Med. **23**, Suppl. 1, 254 (1965a).

— Observations on the effect of parathyroid products on explanted mouse limb-bone rudiments. In: The parathyroid glands (GAILLARD, P.J., TALMAGE, R.V., BUDY, A.M., eds.), p. 145. Chicago: The Univ. of Chicago Press 1965b.

GILL, J.R., JR., BELL, N.H., BARTTER, F.C.: Effect of parathyroid extract on magnesium excretion in man. J. appl. Physiol. **22**, 136 (1967).

GITELMAN, H.J., KUKULI, S., WELT, L.G.: The influence of the parathyroid glands on the hypercalcemia of experimental magnesium depletion in the rat. J. clin. Invest. **47**, 118 (1968).

GOLDBERG, M.F., TASHJIAN, A.H., ORDER, S.E., DAMMIN, G.J.: Renal adenocarcinoma containing a parathyroid hormone-like substance and associated with marked hypercalcemia. Amer. J. Med. **36**, 805 (1964).

HABENER, J.F., POWELL, D., MURRAY, T.M., MAYER, G.P., POTTS, J.T., JR.: Parathyroid hormone: secretion and metabolism in vivo. Proc. nat. Acad. Sci. (USA) **68**, 2986 (1971).

HALVER, B.: The tubular transport of glucose as a measure of parathyroid function. Acta med. scand. **181**, 209 (1967).

HAMILTON, J.W., DELUT, A.C., COHN, D.V.: The effect of parathyroid hormone in vivo on the labeling of the nuclear ribonuclein acids from the intestinal mucosa and liver of vitamin D deficient and normal chicks. Biochim. biophys. Acta (Amst.) **155**, 311 (1968).

HARRISON, H.C., HARRISON, H.E., PARK, E.A.: Vitamin D and citrate metabolism. Proc. Soc. exp. Biol. (N.Y.) **96**, 768 (1957).

HEERSCHE, J.N.M.: Metabolism of bone in tissue culture: the effect of parathyroid hormone and thyrocalcitonin on the process of bone demineralisation. Proc. kon. ned. Akad. Wet. **C72** (1969).

HEKELMAN, J.W.: Bone metabolism and the action of parathyroid extract. Thesis. Leiden (1963).

HERRMANN-ERLEE, M.P.M.: The in vitro effect of parathyroid extract on the amount of NADP in embryonic mouse calvaria. Biochem. biophys. Res. Commun. **23**, 1 (1966).

KEISER, H.R., GILL, J.R., SJOERDSMA, A., BARTTER, F.C.: Relation between urinary hydroxyproline and parathyroid function. J. clin. Invest. **43**, 1073 (1964).

LAMBERT, P.P., CORVILAIN, J.: Site of action of parathyroid hormone and role of growth hormone in phosphate excretion. Mem. Soc. Endocr. **13**, 139 (1963).

LEE, J.B., TASHJIAN, A.H., JR., STREETO, J.M., FRANTZ, A.G.: Familial pseudohypoparathyroidism. New Engl. J. Med. **279**, 1179 (1968).

MACINTYRE, I., BOSS, S., TROUGHTON, V.A.: Parathyroid hormone and magnesium homeostasis. Nature (Lond.) **198**, 1058 (1963).

MARTIN, T.J., MELICK, R.A., LUISE, M.DE: Metabolism of parathyroid hormone. Biochem. J. **111**, 509 (1969).

MASSRY, S.G., COBURN, J.W., KLEEMAN, C.R.: Renal handling of magnesium in the dog. Amer. J. Physiol. **216**, 1460 (1969).

MCDONALD, J.E., MAMOLA, N., HENNEMAN, P.H.: Urinary hydroxyproline as an index of bone resorption. Clin. Res. **13**, 329 (1965).

MELICK, R.A., GILL, J.R., JR., BERSON, S.A., YALOW, R.S., BARTTER, F.C., POTTS, J.T., JR., AURBACH, G.D.: Antibodies and clinical resistance to parathyroid hormone. New Engl. J. Med. **276**, 144 (1967).

— MARTIN, T.J.: Parathyroid hormone metabolism in man: effect of nephrectomy. Clin. Sci. **37**, 667 (1969).

MUNSON, P.L.: Studies on the role of parathyroids in calcium and phosphate metabolism. Ann. N.Y. Acad. Sci. **60**, 776 (1955).

MURPHY, D.L.: Hypercalcemia and gastric secretion in man. J. appl. Physiol. **21**, 1607 (1966).

NAGATA, N., RASMUSSEN, H.: Parathyroid hormone, 3'5'-AMP, Ca^{++}, and renal gluconeogenesis. Proc. nat. Acad. Sci. (Wash.) **65**, 368 (1970).

NEUMAN, W.F., NEUMAN, M.W.: The chemical dynamics of bone mineral. Chicago: Univ. of Chicago Press 1958.

NIALL, H. D., KEUTMANN, H. T., SAUER, R., HOGAN, M., DAWSON, B. F., AURBACH, G. D., POTTS, J. T., JR.: The amino acid sequence of bovine parathyroid hormone. Hoppe-Seylers Z. physiol. Chem. **35**, 1586 (1970).

NICHOLSON, T. F.: The mode and site of the renal action of parathyroid extract in the dog. Canad. J. Biochem. **37**, 113 (1959).

OLDHAM, S. B., FISCHER, J. A., ARNAUD, C. D.: Calcium binding protein from the parathyroid gland. J. Lab. clin. Med., abstract (1971 b).

— — CAPEN, C. C., SIZEMORE, G. W., ARNAUD, C. D.: Dynamics of parathyroid hormone secretion in vitro. Amer. J. Med. **50**, 650 (1971 a).

ORIMO, H., FUJITA, T., MORII, H., NAKAO, K.: Inactivation in vitro of parathyroid extract activity in kidney slices. Endocrinology **76**, 255 (1965).

O'RIORDAN, J. L. H., WOODHEAD, J. S., ROBINSON, C. J., PARSONS, J. A., KEUTMANN, H., NIALL, H., POTTS, J. T., JR.: Structure-function studies in parathyroid hormone. Proc. roy. Soc. Med. **64**, 1263 (1971).

OWEN, M., BINGHAM, P. J.: The effect of parathyroid extract on RNA synthesis in osteogenic cells in vivo. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 216. Amsterdam: Excerpta Medica 1968.

PARK, H. Z., TALMAGE, R. V.: Comparison of the effects of calcium and endogenous parathyroid hormone on RNA synthesis in rat bone. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 203. Amsterdam: Excerpta Medica 1968.

PARSONS, J. A., ROBINSON, C. J.: Calcium shift into bone causing transient hypocalcemia after injection of parathyroid hormone. Nature **230**, 581 (1971).

POTTS, J. T., JR.: Personal communications (1973).

— AURBACH, G. D., SHERWOOD, L. M., SANDOVAL, A.: Structural basis of biological and immunological activity of parathyroid hormone. Proc. nat. Acad. Sci. (Wash.) **54**, 1743 (1965).

— MURRAY, T. M., PEACOCK, M., NIALL, H. D., TREGEAR, G. W., KEUTMANN, H. T., POWELL, D., DEFTOS, L. J.: Parathyroid hormone: Sequence, synthesis, immunoassay studies. Amer. J. Med. **50**, 639 (1971 b).
G. D.: Synthesis of a biologically active N-terminal tetraricontapeptide of parathyroid hormone. Proc. nat. Acad. Sci. (Wash.) **68**, 63 (1971 a).

PULLMAN, T. N., LAVENDER, A. R., AHO, J., RASMUSSEN, H.: Direct renal action of a purified parathyroid extract. Endocrinology **67**, 570 (1960).

RAISZ, L. G.: Bone resorption in tissue culture. Factors influencing the response to parathyroid hormone. J. clin. Invest. **44**, 103 (1965 b).

— Parathyroid gland metabolism. Arch. intern. Med. **124**, 389 (1969).

— AU, W. Y., STERN, P. H.: Regulation of parathyroid activity. In: The parathyroid glands (GAILLARD, P. J., TALMAGE, R. V., BUDY, A. M., eds.). Chicago: Chicago Univ. Press 1965 a.

RASMUSSEN, H.: Parathyroid hormone. Amer. J. Med. **30**, 112 (1961).

— Mitochondrial ion transport: mechanism and physiological significance. Fed. Proc. **25**, 903 (1966).

— ARNAUD, C., HAWKER, C.: Actinomycin D and the response to parathyroid hormone. Science **144**, 1019 (1964 a).

— CRAIG, L. C.: Purification of parathyroid hormone by use of countercurrent distribution. J. Amer. chem. Soc. **81**, 5003 (1959).

— DELUCA, H. F.: Calcium homeostasis. Ergebn. Physiol. **53**, 108 (1963 a).

— — ARNAUD, C., HAWKER, C., STEDINGK, M.: The relationship between vitamin D and parathyroid hormone. J. clin. Invest. **42**, 1940 (1963 b).

— FEINBLATT, J., NAGATA, N., PECHET, M.: Effect of ions upon bone cell function. Fed. Proc. **29**, 1190 (1970).

— PECHET, M., FAST, D.: Effect of dibutyryl cyclic adenosine 3′,5′-monophosphate, theophylline, and other nucleoticles upon calcium and phosphate metabolism. J. clin. Invest. **47**, 1843 (1968).

— SHIRASU, H., OGATA, E., HAWKER, C.: Parathyroid hormone and mitochondrial metabolism. J. biol. Chem. **242**, 4669 (1967 a).

— SZE, Y. L., YOUNG, R.: Further studies on the isolation and characterisation of parathyroid hormone. J. biol. Chem. **239**, 2852 (1964 b).

— TENENHOUSE, A.: Thyrocalcitonin, osteoporosis and osteolysis. Amer. J. Med. **43**, 711 (1967 b).

REISS, E., CANTERBURY, J. M.: A radioimmunoassay for parathyroid hormone in man. Proc. Soc. exp. Biol. (N.Y.) **128**, 501 (1968 a).

— — Application of radioimmunoassay to differentiation of adenoma and hyperplasia and to preoperative localization of hyperfunctioning parathyroid glands. New Engl. J. Med. **280**, 1381 (1969).

REYNOLDS, J. J.: Inhibition of calcitonin of bone resorption induced in vitro by Vitamin A. Proc. roy. Soc. B **170**, 61 (1968).

RIGGS, B. L., ARNAUD, C. D., REYNOLDS, J. C., SMITH, L. H.: Immunologic differentiation of primary hyperparathyroidism from hyperparathyroidism due to nonparathyroid cancer. J. clin. Invest. **50**, 2079 (1971).

— KELLY, P., JOWSEY, J., KEATING, F. R., JR.: Skeletal alterations in hypoparathyroidism: determination of bone formation, resorption and morphologic changes by microradiography. J. clin. Endocr. **25**, 777 (1965).

ROSENBAUM, J. L., RAMIREZ, O., SAMUELS, S., BREST, A. N., MOYER, J. H.: Effect of sudden blood calcium depletion on phosphorus excretion. Endocrinology **74**, 266 (1964).

RUSSELL, R. G. G., CASEY, P. A., BISAZ, S., FLEISCH, H.: Further studies on the role of pyrophosphate in mineralized tissues and on the function of phosphates. In: Les tissus calcifiés, p. 65. Paris: Soc. d'Ed. d'Einseignement sup., Bordeaux 1968 a.

— — FLEISCH, H.: Stimulation of phosphate excretion by renal arterial infusion of 3′5′-AMP (cyclic AMP). — A possible mechanism of action of parathyroid hormone. Calc. Tiss. Res. **2**, Suppl. 54 (1968 b).

SCHMID, J. R., LABHART, A., ROSSIER, P. H.: Relationship of multiple endocrine adenomas to the syndrome of ulcerogenic islet cell adenomas (Zollinger-Ellison). Amer. J. Med. **31**, 343 (1961).

SHERWOOD, L. M., HERRMAN, I., BASSET, C. W.: Parathyroid hormone secretion in vitro: regulation by calcium and magnesium ions. Nature **225**, 1056 (1970).

— MAYER, G. P., RAMBERG, C. F., JR., KRONFELD, D. S., AURBACH, G. D., POTTS, J. T., JR.: Regulation of parathyroid hormone secretion. Endocrinology **83**, 1043 (1968 a).

— O'RIORDAN, J. L. H., AURBACH, G. D., POTTS, J. T., JR.: Production of parathyroid hormone by nonparathyroid tumors. J. clin. Endocr. **27**, 140 (1967).

— POTTS, J. T., JR., CARE, A. D., MAYER, G. P., AURBACH, G. D.: Evaluation by radioimmunoassay of factors controlling the secretion of parathyroid hormone. Nature (Lond.) **209**, 52 (1966).

— RODMAN, J. S., LUNDBERG, W. B., JR.: Evidence for a precursor to circulating parathyroid hormone. Proc. nat. Acad. Sci. (Wash.) **67**, 1631 (1970).

SIEGMUND, P., BAUDITZ, W.: Die Wirkung von Azetazolamid (Diamox) auf den Anstieg der Plasma Calciumwerte nach Parathormon. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **251**, 288 (1965).

SUSI, F. R., GOLDHABER, P., JENNINGS, J. M.: Histochemical and biochemical study of acid phosphatase in resorbing bone in culture. Amer. J. Physiol. **211**, 959 (1966).

TALMAGE, R. V., DOTY, S. B., COOPER, C. W., YATES, C., NEUENSCHWANDER, J.: Cytological and biochemical changes resulting from fluctuations in endogenous parathyroid

hormone levels. In: The parathyroid glands (GAILLARD, P.J., TALMAGE, R.V., BUDY, A.M., eds.), p. 107. Chicago: Univ. of Chicago Press 1965.

TENENHOUSE, A., MEIER, R., RASMUSSEN, H.: Parathyroid hormone and bone mobilisation in vitro. J. biol. Chem. **241**, 1314 (1966).

TRUDEAU, W.L., MCGUIGAN, J.E.: Effects of calcium on serum gastric levels in the Zollinger-Ellison syndrome. New Engl. J. Med. **281**, 862 (1969).

VAES, G.: La résorption osseuse et l'hormone parathyroidienne. Bruxelles: Visscher 1967.

WALKER, D.G., LAPIERE, C.M., GROSS, J.: A collagenolytic factor in rat bone promoted by parathyroid extract. Biochem. biophys. Res. Commun. **15**, 397 (1964).

WEBSTER, G.D., MANN, J.B., HILLS, A.G.: The effect of phosphate infusions upon renal phosphate clearance in man. Evidence for tubular phosphate secretion. Metabolism **16**, 797 (1967).

WHITEHEAD, R.G., WERDMANN, S.M.: The effect of parathormone on the uptake of ^{32}P into adenosine triphosphate and bone salt in kittens. Biochem. J. **71**, 312 (1959).

WINTER, M., MORAVA, E., SIMON, G., SÓS, J.: The role of the parathyroid glands in the absorption of calcium from the small intestine. J. Endocr. **47**, 65 (1970).

YOUNG, R.W.: Specialisation of bone cells. Bone biodynamics (H.M. FROST, ed.). Boston: Little, Brown 1964.

ZOLLINGER, R.M., ELLISON, E.H.: Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. Ann. Surg. **142**, 709 (1955).

Calcitonin (Thyrocalcitonin)

ALIAPOULIOS, M.A., GOLDHABER, F., MUNSON, P.L.: Thyrocalcitonin inhibition of bone resorption induced by parathyroid hormone in tissue culture. Science **151**, 330 (1966).

ARDAILLOU, R., VUAGNAT, P., MILHAUD, G., RICHET, G.: Effets de la thyrocalcitonine sur l'excretion rénale des phosphates, du calcium et des ions H^+ chez l'homme. Nephron **4**, 298 (1967).

ARNAUD, C.D., TSAO, H.S., LITTLEDIKE, T.: Calcium homeostasis, parathyroid hormone, and calcitonin. Proc. Mayo Clin. **45**, 125 (1970).

AVIOLI, L.V., BIRGE, S.J., SCOTT, S., SHIEBER, W.: The role of the thyroid gland during glucagon-induced hypocalcemia in the dog. Amer. J. Physiol. **216**, 939 (1969).

BAUM, J.L.: Abnormal intradermal histamine reaction in the syndrome of phaeochromocytoma, medullary carcinoma of the thyroid gland and multiple mucosal neuromas. New Engl. J. Med. **284**, 963 (1971).

BAYLIN, S.B., BEAVEN, M.A., ENGELMAN, K., SJOERDSMA, A.: Elevated histaminase in medullary carcinoma of the thyroid gland. New Engl. J. Med. **283**, 1239 (1970).

BELL, N.H.: Effects of glucagon, dibutyryl cyclic 3′,5′-adenosine monophosphate, and theophylline on calcitonin secretion in vitro. J. clin. Invest. **49**, 1368 (1970).

BIJOVET, O.L.M., SLUYS VEER, J. VAN DER, JANSEN, A.P.: Effects of calcitonin on patients with Paget's disease, thyrotoxicosis, or hypercalcemia. Lancet **1968 I**, 876.

BINSWANGER, U., FISCHER, J.A., MERZ, W., SCHENK, R., MAYOR, G., UEHLINGER, E.: Thyreocalcitonin und primärer Hyperparathyreoidismus. Analyse knochenbioptischer Befunde. 14. Symposium Endokrinologie (1968), S. 95.

BUSSOLATTI, G., PEARSE, A.G.E.: Immunofluorescent localisation of calcitonin in the "C" cells of pig and dog. J. Endocr. **37**, 205 (1967).

CAMERON, D.A.: Fine structure and function in thyroid "C" cells and parathyroid cells. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 437. Amsterdam: Excerpta Medica 1968.

CARE, A.D., BATES, R.F.M., GITELMAN, H.J.: The role of glucagon in the release of thyrocalcitonin. J. Endocr. **43** (1969).

— COOPER, C.W., DUNCAN, T., ORIMO, H.: A study of thyrocalcitonin secretion by direct measurements of in vivo secretion rates in pigs. Endocrinology **83**, 161 (1968).

— SHERWOOD, L.M., POTTS, J.T., JR., AURBACH, G.D.: Perfusion of isolated parathyroid gland of the goat and sheep. Nature (Lond.) **209**, 55 (1966).

CARVALHEIRA, A.F., PEARSE, A.G.E.: Cytochemical evidence for the ultimobranchial origin of the "C" cells in rodent thyroid. In: Calcitonin, p. 122. London: Heinemann 1968.

CLARK, J.D., KENNY, A.D.: Hog thyrocalcitonin in the dog: urinary calcium, phosphorus, magnesium, and sodium respondes. Endocrinology **84**, 1199 (1969b).

CLARK, M.B., BOYD, G.W., BYFIELD, P.G.H., FOSTER, G.V.: A radioimmunoassay for human calcitonin-M. Lancet **1969a II**, 74.

COPP, D.H., CAMERON, E.C., CHENEY, B.A., DAVIDSON, A.G.F., HENZE, K.G.: Evidence for calcitonin. — A new hormone from the parathyroid that lowers blood calcium. Endocrinology **70**, 638 (1962).

— KUCZERPA, A.V.: Effect of age and growth on the response to thyrocalcitonin in the rat. In: Les tissues calcifiés, p. 167. Paris: Soc. d'Ed. d'Einseignement sup. (1968).

CUNLIFFE, W.J., BLACK, M.M., HALL, R., JOHNSTON, J.D.A., HUDGSON, P., SHUSTER, S., GUDMANDSSON, T.V., JOPLIN, G.F., WILLIAMS, E.D., WOODHOUSE, N.J.Y., GALANTE, L., MACINTYRE, I.: A calcitonin-secreting thyroid carcinoma. Lancet **1968 II**, 63.

— HUDGSON, P., FULTHORPE, J.J., BLACK, M.M., HALL, R., JOHNSTON, I.D.A., SHUSTER, S.: A calcitonin-secreting medullary thyroid carcinoma associated with mucosal neuromas, Marfanoid features, myopathy and pigmentation. Amer. J. Med. **48**, 120 (1970).

EVANSON, J.M., GARNER, A., HOLMES, A.M., LUMB, G.A., STANBURY, S.W.: Interrelations between thyrocalcitonin and parathyroid hormone in rats. Clin. Sci. **32**, 271 (1967).

FISCHER, J.A., BINSWANGER, U., FREY-WETTSTEIN, M.: "Hypoparathyroidism", hyperostosis, normophosphatemia and thyrocalcitonin. Acta endocr. (Kbh.) **55**, 562 (1967).

— OLDHAM, S.B., SIZEMORE, G.W., ARNAUD, C.D.: Calcitonin stimulation of parathyroid hormone secretion in vitro. Horm. Metab. Res. **3**, 223 (1971).

FOSTER, G.V., BAGHDIANTZ, A., KUMAR, M.A., SLACK, E., SOLIMAN, H.A., MACINTYRE, I.: Thyroid origin of calcitonin. Nature (Lond.) **202**, 1303 (1964).

— DOYLE, F.H., BORDIER, P., MATRAJT, H.: Effect of thyrocalcitonin on bone. Lancet **1966 II**, 1428 (a).

— JOPLIN, G.F., MACINTYRE, I., MELVIN, K.E.M., SLACK, E.: Effect of thyrocalcitonin in man. Lancet **1966 I**, 107 (b).

FRIEDMAN, J., AU, W.Y.W., RAISZ, L.G.: Responses of fetal bone to thyrocalcitonin in tissue culture. Endocrinology **82**, 149 (1968).

GALANTE, L., GUDMUNDSSON, T.V., MATTHEWS, E.T., TSE, A., WILLIAMS, E.D., WOODHOUSE, N.J.Y., MACINTYRE, I.: Thymic and parathyroid origin of calcitonin in man. Lancet **1968 II**, 537.

GITTES, R.F., TOVERUD, S.U., COOPER, C.W.: Effects of hypercalcemia on the thyrocalcitonin content of rat thyroid glands. Endocrinology **82**, 83 (1968).

GRAY, T.K., MUNSON, P.L.: Thyrocalcitonin: evidence for physiological function. Science **166**, 512 (1969).

GUDMUNDSSON, T.V., MACINTYRE, I., SOLIMAN, H.A.: The isolation of thyrocalcitonin and a study of its effects in the rat. Proc. roy. Soc. B **164**, 460 (1966).

— WOODHOUSE, N.J.Y., OSATO, T.D., GALANTE, L., MATTHEWS, E.W., MACINTYRE, I., KENNY, A.D., WIGGINS, R.C.: Plasmacalcitonin in man. Lancet **1969 I**, 443.

GUTTMANN, ST., PLESS, J., SANDRIN, E., JAQUENOD, P.-A., POSSENT, H., WILLEMS, H.: Synthese des Thyreocalcitonins. Helv. chim. Acta **51**, 1155 (1968).

HAAS, H.G., DAMBACHER, M.A.: Thyrocalcitonin in man. Helv. med. Acta **34**, 327 (1968).

— — Guncaga, J., Lauffenburger, T.: Renal effects of calcitonin and parathyroid extract in man. Studies in hypoparathyroidism. J. clin. Invest. **50**, 2689 (1971).

Habener, J.F., Singer, F.R., Deftos, L.J., Neer, R.M., Potts, J.T., Jr.: Explanation for unusual potency of salmon calcitonin. Nature New Biol. **232**, 91 (1971).

Hachmeister, U., Bönicke, J., Lenke, M., Kracht, J.: Die C-Zellen der Rattenschilddrüse bei Hyper- und Hypocalcämie. 13. Symp. Endokrinologie. Berlin-Heidelberg-New York: Springer 1967.

Harrison, H.C., Harrison, H.E., Park, E.A.: Vitamin D and citrate metabolism. Proc. Soc. exp. Biol. (N.Y.) **96**, 768 (1957).

Hirsch, P.F.: Enhancement of hypocalcemic activity of thyrocalcitonin by inorganic phosphate. In: Calcitonin 1967, p. 11. London: Heinemann 1968.

— Gauthier, G.F., Munson, P.L.: Thyroid hypocalcemic principle and recurrent laryngeal nerve injury as factors affecting the response to parathyroidectomy in rats. Endocrinology **73**, 244 (1963).

Kaplan, E.L., Arnaud, C.D., Hill, B.J., Peskin, G.W.: Adrenal medullary calcitonin-like factor: a key to multiple endocrine neoplasia, type 2? Surgery **68**, 146 (1970).

Kenny, A.D., Heiskell, C.A.: Effect of crude thyrocalcitonin on calcium and phosphorus metabolism in rats. Proc. Soc. exp. Biol. (NY) **120**, 269 (1965).

Keutmann, H., Parsons, J.A., Potts, J.T., Jr., Schlueter, R.J.: Isolation and chemical properties of two calcitonins from salmon ultimobranchial glands. J. biol. Chem. **245**, 1491 (1970).

Keynes, W.M., Till, A.S.: Medullary carcinoma of the thyroid gland. Quart. J. Med. NS **40**, 443 (1971).

Klein, D.C., Talmage, R.V.: Thyrocalcitonin suppression of hydroxyproline release from bone. Proc. Soc. exp. Biol. (N.Y.) **127**, 95 (1968).

Kohler, H.F., Pechet, M.M.: The inhibition of bone resorption by thyrocalcitonin. J. clin. Invest. **45**, 1033 (1966).

Lee, M.R., Deftos, L.J., Potts, J.T., Jr.: Control of secretion of thyrocalcitonin in the rabbit as evaluated by immunoassay. Endocrinology **84**, 36 (1969).

— Tashjian, A.H., Jr., Streeto, J.M., Frantz, A.G.: Familial pseudohypoparathyroidism. New Engl. J. Med. **279**, 1179 (1968).

MacIntyre, I.: Calcitonin: A general review. Calc. Tiss. Res. **1**, 173 (1967).

Martin, T.J., Robinson, C.J., MacIntyre, I.: The mode of action of thyrocalcitonin. Lancet **1966I**, 900.

Mazzuoli, G.F., Terrenato, L., Coen, G.: Calcium homeostasis in hypothyroidism and hypoparathyroidism evaluated by means of calcium infusion. 4th European Symposium on calcified tissues, p. 71. Amsterdam: Excerpta Medica 1966.

Melvin, K.E.W., Miller, H.H., Tashjian, A.H., Jr.: Early diagnosis of medullary carcinoma of the thyroid gland by means of calcitonin assay. New Engl. J. Med. **285**, 1115 (1971).

Milhaud, G., Moukhtar, M.S.: Hypophysectomie et thyrocalcitonine. C. R. Acad. Sci. (Paris) **260**, 3179 (1965).

Neher, R., Riniker, B., Rittel, W., Zuber, H.: Menschliches Calcitonin. Struktur von Calcitonin M und D. Helv. chim. Acta **51**, 1900 (1968).

Parsons, J.A., Robinson, C.J.: The effect of thyrocalcitonin on isolated perfused bone. In: Calcitonin 1967, p. 256. London: Heinemann 1968.

Pearse, A.G.E.: The cytochemistry of the thyroid C cells and their relationship to calcitonin. Proc. roy. Soc. B **164**, 478 (1966).

Raisz, L.G., Au, W.Y., Friedman, J., Niemann, I.: Thyrocalcitonin and bone resorption. Amer. J. Med. **43**, 684 (1967).

Rasmussen, H., Anast, C., Arnaud, C.: Thyrocalcitonin, EGTA, and urinary electrolyte excretion. J. clin. Invest. **46**, 746 (1967).

Rittel, W., Brugger, M., Kamber, B., Riniker, B., Sieber, P.: Die Synthese des α-Thyreocalcitonins. Helv. chim. Acta **51**, 924 (1968).

Robinson, C.J., Martin, T.J., Matthews, E.W., MacIntyre, I.: Mode of action of thyrocalcitonin. J. Endocr. **39**, 71 (1967).

— Matthews, E.W., MacIntyre, I.: The effect of parathyroid hormone and thyrocalcitonin on the intestinal absorption of calcium and magnesium. In: Les tissues calcifiés, p. 279. Paris: Soc. d'Ed. d'Einseignement sup. (1968).

Sieber, P., Brugger, M., Kamber, B., Riniker, B., Rittel, W.: Menschliches Calcitonin. Die Synthese von Calcitonin M. Helv. chim. Acta **51**, 2057 (1968).

Singer, F.R., Woodhouse, N.J.Y., Parkinson, D.K., Joplin, D.F.: Some acute effects of administered porcine calcitonin in man. Clin. Sci. **37**, 181 (1969).

Sturtridge, W.C., Kumar, M.A.: Assay of calcitonin in human plasma. Lancet **1968I**, 725.

Talmage, R.V., Neuenschwander, J., Kraintz, L.: Evidence for the existence of thyrocalcitonin in the rat. Endocrinology **76**, 107 (1965).

Tashjian, A.H., Jr.: Immunoassay of thyrocalcitonin. I. The method and its serological specificity. Endocrinology **84**, 140 (1969).

— Howland, B.G., Melvin, K.E.W., Hill, C.S.: Immunoassay of human calcitonin. New Engl. J. Med. **283**, 890 (1970).

— Melvin, K.E.W.: Medullary carcinoma of the thyroid gland. New Engl. J. Med. **279**, 279 (1968).

Walker, D.G.: Counteraction to parathyroid extract in osteopetrotic mice as revealed in the plasma calcium level and ability to incorporate H-proline into bone. Endocrinology **79**, 836 (1966).

Wallach, S., Bellavia, J.V., Schorr, J.: Tissue distribution of electrolytes, ^{47}Ca and ^{28}Mg in experimental hyper- and hypoparathyroidism. Endocrinology **78**, 16 (1966).

White, E., Ahmann, T.M.: Calcitonin activity in hereditary osteopetrosis. J. clin. Invest. **44**, 1111 (1965).

Williams, G.A., Hatgis, G.K., Galloway, W.B., Henderson, W.J.: Evidence for thyrocalcitonin in man. Proc. Soc. exp. Biol. (N.Y.) **122**, 1273 (1966).

Vitamin D

Albright, F., Reifenstein, E.C., Jr.: The parathyroid glands and metabolic bone diseases. Baltimore: Williams & Wilkins 1948.

Bayard, F., Bec, P., Louvet, J.P.: Measurment of plasma 25-hydroxycholecalciferol in man. Europ. J. clin. Invest. **2**, 195 (1972).

Bauer, G.C.H., Carlsson, A., Lindquist, B.: Bone salt metabolism in human rickets studied with radioactive phosphorus. Metabolism **5**, 573 (1956).

Bélanger, L.F.: Osteolysis: an outlook on its mechanism and its causation. In: The parathyroid glands, p. 137. Chicago: Univ. of Chicago Press 1965.

Bell, N.H., Gill, J.R., Jr., Bartter, F.C.: On the abnormal calcium absorption in sarcoidosis. Amer. J. Med. **36**, 500 (1964).

Belsey, R., DeLuca, H.F., Potts, J.T., Jr.: Competitive binding assay for vitamin D and 25-OH vitamin D. J. clin. Endocr. **33**, 554 (1971).

Binswanger, U., Fischer, J.A., Schenk, R., Merz, W.: Osteophathie bei chronischer Niereninsuffizienz. Dtsch. med. Wschr. **96**, 1914 (1971).

Bordier, P., Matrajt, H., Hioco, D., Hepner, G.W., Thompson, G.E., Booth, C.C.: Subclinical vitamin D deficiency following gastrectomy. Lancet **1968I**, 437.

BRICKMAN, A.S., COBURN, J.W., NORMAN, A.W.: Action of 1,25-dihydroxycholecaliferol, a potent, kidney-produced metabolite of vitamin D_3, in uremic man. New Engl. J. Med. **287**, 891 (1972).

CARLSSON, A.: Tracer experiments on the effects of vitamin D on skeletal metabolism of calcium and phosphorus. Acta physiol. scand. **26**, 212 (1952).

— HOLLUNGER, E., LINDQUIST, M., MAGNUSSON, T.: The effect of vitamin D on the citric acid metabolism. Acta physiol. scand. **31**, 317 (1954).

CORRADINO, R.A., WASSERMANN, R.H.: Actinomycin D inhibition of vitamin-D_3 induced calcium-bindung protein formation in chick duodenal mucosa. Arch. Biochem. **126**, 957 (1968).

CRUESS, R.L., CLARK, J.: Effect of hypervitaminosis D upon the phospholipids of metaphyseal and diaphyseal bone. Proc. Soc. exp. Biol. (N.Y.) **126**, 8 (1967).

DELUCA, H.F.: The functional metabolism of vitamin D. In: Calcium, parathyroid hormone and the calcitonins. TALMAGE, R.V., MUNSON, P.L. (eds.), p. 221. Amsterdam: Excerpta Medica 1973.

— WELLER, M., BLUNT, J.W., NEVILLE, P.F.: Synthesis, biological activity, and metabolism 22,23-^{3}H-vitamin D_4. Arch. Biochem. **124**, 122 (1968).

EISENBERG, E.: Effects of serum calcium level and parathyroid extracts in phosphate and calcium excretion in hypoparathyroid patients. J. clin. Invest. **44**, 942 (1965).

ENGSTROM, G.W., DELUCA, H.F.: Vitamin D-stimulated release of calcium from mitochondria. Biochemistry **3**, 203 (1964).

FELLERS, F.X., SCHWARTZ, R.: Etiology of the severe form of idiopathic hypercalcemia of infancy. New Engl. J. Med. **259**, 1050 (1958).

FLANAGAN, B., NICHOLS, G., JR.: Metabolic studies in human bone in vitro. II. Changes in hyperparathyroidism. J. clin. Invest. **44**, 1795 (1965).

FRASER, R., HARRISON, M., IBBERTSON, K.: The rate of calcium turnover in bone. Quart. J. Med., N.S. **29**, 85 (1960).

— KODICEK, E.: Unique biosynthesis by kidney of a biologically active vitamin D metabolite. Nature **228**, 764 (1970).

FROST, H.M.: Bone remodelling dynamics. Springfield: Ch.C. Thomas 1963.

GARNER, A., BALL, J.: Quantitative observations on mineralised and unmineralised bone in chronic renal azotaemia and intestinal malabsorption syndrome. J. Path. Bact. **91**, 545 (1966).

GOLDHABER, P.: Bone-resorption factors, cofactors, and giant vacuole osteoclasts in tissue culture. The parathyroid glands (GAILLARD, P.J., TALMAGE, R.V., BUDY, A.M., eds.), p. 153. Chicago: University of Chicago Press 1965.

HARRIS, F., HOFFENBERG, R., BLACK, E.: Calcium kinetics in vitamin D deficiency rickets. I. Plasma kinetic studies after intraveneous and oral ^{47}Ca. Metabolism **14**, 1101 (1965).

HARRISON, H.E., HARRISON, H.C.: Renal excretion of inorganic phosphate in relation to the action of vitamin D and parathyroid hormone. J. clin. Invest. **20**, 47 (1941).

— — Intestinal transport of phosphate: action of vitamin D, calcium and potassium. Amer. J. Physiol. **201**, 1007 (1961).

— — Vitamin D and permeability of intestinal mucosa to calcium. Amer. J. Physiol. **208**, 370 (1965).

— — Actinomycin D inhibition of intestinal transport of calcium and of vitamin D action. Proc. Soc. exp. Biol. (N.Y.) **121**, 312 (1966).

— — Comparison between crystalline dihydrotachysterol and calciferol in patients requiring pharmacologic vitamin D therapy. New Engl. J. Med. **276**, 894 (1967).

IRVING, J.R.: A histological stain for newly calcified tissues. Nature (Lond.) **181**, 704 (1959).

KIMBERG, D.V.: Effects of vitamin D and steroid hormones on the active transport of calcium by the intestine. New Engl. J. Med. **280**, 1396 (1969).

KOWARSKI, S., SCHACHTER, D.: Effects of vitamin D on phosphate transport and incorporation into mucosal constituents of rat intestinal mucosa. J. biol. Chem. **244**, 211 (1969).

LIFSHITZ, F., HARRISON, H.C., HARRISON, H.E.: Effects of vitamin D in magnesium metabolism in rats. Endocrinology **81**, 849 (1967).

MAWER, E.B., STANBURY, S.W.: Metabolism of vitamin D_3 in man. Biochem. J. **110**, 53P (1968).

MELANCON, M.J., DELUCA, H.F.: Vitamin D stimulation of calcium-dependent adenosine triphosphatase in chick intestinal brush borders. Biochemistry **9**, 1658 (1970).

OLSON, E.B., DELUCA, H.F.: 25-Hydroxycholecalciferol: direct effect on calcium transport. Science **165**, 405 (1969).

PATERSON, C.R., FOURMAN, P.: Collagen synthesis and carbohydrate metabolism of rachitic bone. Biochem. J. **109**, 101 (1968).

PECHET, M.M., BOBADILLA, E., CARROLL, E.L., HESSE, R.H.: Regulation of bone resorption and formation. Amer. J. Med. **43**, 696 (1967).

PONCHON, G., DELUCA, H.F.: The role of the liver in the metabolism of vitamin D. J. clin. Invest. **48**, 1273 (1969).

PUSCHETT, J.B., FERNANDEZ, P.C., BOYLE, J.T., GRAY, R.W., OMDAHL, J.L., DELUCA, H.F.: The acute renal tubular effects of 1,25-dihydroxycholecalciferol. Proc. Soc. exp. Biol. Med. **141**, 379 (1972).

RAISZ, L.G., TRUMMEL, C.L., HOLICK, M.F., DELUCA, H.F.: 1,25-dihydroxycholeacalciferol: a potent stimulator of bone resorption in tissue culture. Science **175**, 768 (1972).

RASMUSSEN, H., DELUCA, H.F., ARNAUD, C., HAWKER, C., STEDINGK, M.: The relationship between vitamin D and parathyroid hormone. J. clin. Invest. **42**, 1940 (1963).

— WONG, M., BIKLE, D., GOODMAN, D.B.P.: Hormonal control of the renal conversion of 25-hydroxycholecalciferol, in 1,25-dihydroxycholecalciferol. J. clin. Invest. **51**, 2502 (1972).

ROHR, H.: Autoradiographische Untersuchungen über den Kollagenstoffwechsel bei der experimentellen Rattenrachitis. Z. ges. exp. Med. **139**, 621 (1965).

SCHACHTER, D., KIMBERG, D.V., SCHENKER, H.: Active transport of calcium by intestine: action and bioassay of vitamin D. Amer. J. Physiol. **200**, 1263 (1961).

— KOWARSKI, S., FINKELSTEIN, J.D., WONG, M.R.: Tissue concentration differences during active transport of calcium by intestine. Amer. J. Physiol. **211**, 1131 (1966).

SMITH, J.E., GOODMAN, D.S.: The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. J. clin. Invest. **50**, 2159 (1971).

TANAKA, Y., DELUCA, H.F.: The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. Arch. Biochem. Biophys. **154**, 566 (1973).

TAYLOR, R.L., LYNCH, H.J., JR., WYSOR, W.E., JR.: Seasonal influence of sunlight on the hypercalcemia of sarcoidosis. Amer. J. Med. **34**, 221 (1963).

THOMAS, W.C., JR., MORGAN, H.E., CONNOR, T.B., HADDECK, L., BILLS, C.E., HOWARD, J.E.: Studies of antirachitic activity in sera from patients with disorders of calcium metabolism. J. clin. Invest. **38**, 1078 (1959).

TSAI, H.C., WONG, R.G., NORMAN, A.W.: Studies on calciferol metabolism. IV. Subcellular localization of 1,25-dihydroxy-vitamin D_3 in intestinal mucosa and correlation with increased calcium transport. J. biol. Chem. **247**, 5511 (1972).

WALLING, M.W., ROTHMAN, S.S.: Phosphate-independent, carrier-mediated active transport of calcium by rat intestine. Amer. J. Physiol. **217**, 1144 (1969).

WASSERMANN, R.H.: Calcium transport by the intestine: a model and comment on vitamin D action. Calc. Tiss. Res. **2**, 301 (1968a).

— TAYLOR, A. N.: Vitamin D-dependent calcium-binding protein. J. biol. Chem. **243**, 3987 (1968b).
WERNLY, M.: Die Osteomalazie. Stuttgart: Georg Thieme 1952.

Bone, Calcium, and Phosphate

ARNAUD, C., RASMUSSEN, H., ANAST, C.: Further studies on the interrelationship between parathyroid hormone and vitamin D. J. clin. Invest. **45**, 1955 (1966).
AVIOLI, L. V., BIRGE, S. J., SCOTT, S., SHIEBER, W.: The role of the thyroid gland during glucagon-induced hypocalcemia in the dog. Amer. J. Physiol. **216**, 939 (1969).
BAYLINK, D., WERGEDAL, J., STAUFFER, M.: Formation, mineralization, and resorption of bone in hypophysphatemic rats. J. clin. Invest. **50**, 2519 (1971).
BINSWANGER, U., FEIST, E., RICH, C.: Bone resorption without parathyroid hormone: influence of a low phosphorus diet. Z. ges. exp. Med. **156**, 61 (1971).
— UEHLINGER, E.: Zur Regulation des Serumcalciumspiegel bei chronischer Pyelonephritis. Dtsch. Arch. klin. Med. **213**, 48 (1966).
CARE, A. D., BATES, R. F. M., GITELMAN, H. J.: The role of glucagon in the release of thyrocalcitonin. J. Endocr. **43** (1969).
COBURN, J. W., MASSRY, S. G.: Changes in serum and urinary calcium during phosphate depletion: studies on mechanism. J. clin. Invest. **49**, 1073 (1970).
COPP, D. H.: Endocrine regulation of calcium metabolism. Ann. Rev. Physiol. **32**, 61 (1970).
DELUCA, H. F., MORII, H., MELANCON, M. J., JR.: The interaction of vitamin D, parathyroid hormone and thyrocalcitonin. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 448. Amsterdam: Excerpta Medica 1968.
EISENBERG, E.: Renal effects of parathyroid hormone. In: Parathyroid hormone and thyrocalcitonin (calcitonin), p. 465. Amsterdam: Excerpta Medica 1968.
— Effect of intravenous phosphate on serum strontium and calcium. New Engl. J. Med. **282**, 289 (1969).
FEINBLATT, J., BÉLANGER, L. F., RASMUSSEN, H.: Effect of phosphate infusion on bone metabolism and parathyroid hormone action. Amer. J. Physiol. **218**, 1624 (1970).
FERNLEY, H. N., BISAZ, S.: Studies on alkaline phosphatases: phosphorylation of calf intestinal alkaline phosphatase by ^{32}P-labeled pyrophosphate. Biochem. J. **107**, 279 (1968).
FLEISCH, H., RUSSELL, R. G. G.: Pyrophosphate, polyphosphates and phosphatase. Encyclopedia of pharmacology (H. RASMUSSEN, ed.). New York: Pergamon Press 1970.
— — FRANCIS, M. D.: Diphosphonates inhibit hydroxyapatite dissolution in vitro and bone resorption in tissue culture and in vivo. Science **165**, 1262 (1969).
— — STRAUMANN, F.: Effect of pyrophosphate on hydroxyapatite and its implications in calcium homeostasis. Nature (Lond.) **212**, 901 (1966).
GOLDSMITH, R. S., WOODHOUSE, C. F., INGBAR, S. H., SEGAL, D.: Effect of phosphate supplements in patients with fractures. Lancet **1967 I**, 687.
GUDMUNDSON, T. V., MACINTYRE, I., SOLIMAN, H. A.: The isolation of thyrocalcitonin and a study of its effects in the rat. Proc. roy. Soc. B **164**, 460 (1966).
HERBERT, L. A., LEMON, J., JR., PETERSON, J. R., LEMON, E. J.: Studies of the mechanism by which phosphate infusion lowers serum calcium concentration. J. clin. Invest. **45**, 1886 (1966).
KISTLER, H. J.: Treatment of hypercalcemia with inorganic phosphate. Helv. med. Acta **33**, 447 (1966).
LAVENDER, A. R., PULLMAN, T. N.: Changes in inorganic phosphate excretion induced by renal arterial infusion of calcium. Amer. J. Physiol. **203**, 1025 (1963).
LOTZ, M., ZISMAN, E., BARTTER, F. C.: Phosphorus depletion syndrome in man. New Engl. J. Med. **278**, 409 (1968).
LUDWIG, G. D., KYLE, G. C., BLANCO, M. DE: Tertiary hyperparathyroidism induced by osteomalacia resulting from phosphorus depletion. Amer. J. Med. **43**, 136 (1967).
MCLEAN, F. C., CHRIST, M. C.: Bone, an introduction to the physiology of skeletal tissue. Chicago: University Chicago Press 1961.
NAGENT DE DEUXCHAISNES, C., CRANE, S. M.: The treatment of adult phosphate diabetes and Fanconi syndrome with neutral sodium phosphate. Amer. J. Med. **43**, 508 (1967).
NORDIN, B. E. C., PEACOCK, M.: The rolle of the kidney in serum calcium homeostasis. In: Calcitonin 1969, TALOR, S. (ed.), p. 472. London: W. Heinemann 1970.
RAISZ, L. G., NIEMANN, J.: Effect of phosphate, calcium and magnesium on bone resorption and hormonal responses in tissue culture. Endocrinology **85**, 446 (1969).
— — Effect of phosphate, calcium and magnesium on bone resorption and hormonal response in tissue culture. Endocrinology **85**, 3 (1969).
RASMUSSEN, H., TENENHOUSE, A.: Thyrocalcitonin, osteoporosis and osteolysis. Amer. J. Med. **43**, 711 (1967).
RUSSELL, R. G. G., FLEISCH, H.: Inorganic pyrophosphate and pyrophosphatases in calcification and calcium homeostasis. Clin. Orthop. **69**, 101 (1970).
SHERWOOD, L. M., MAYER, G. P., RAMBERG, C. F., JR., KRONFELD, D. S., AURBACH, G. D., POTTS, J. T., JR.: Regulation of parathyroid hormone secretion. Endocrinology **83**, 1043 (1968).
STERN, P. H., BELL, N. H.: Effects of glucagon on serum calcium in the rat and on bone resorption in tissue culture. Endocrinology **87**, 111 (1970).
TASHJIAN, A. H., JR.: Homeostasis of plasma calcium: effects of actinomycin D, parathyroidectomy and thyrocalcitonin. Endocrinology **77**, 375 (1965).
— Effects of parathyroidectomy and cautery of the thyroid gland on the plasma calcium level of rats with autotransplanted parathyroid glands. Endocrinology **78**, 1144 (1966).

Hypoparathyroidism

Etiology and Pathogenesis

ARSDEL, P. P., JR., VAN: Maternal hyperparathyroidism as a cause of neonatal tetany; case report and review of 29 cases of parathyroid adenoma. J. clin. Endocr. **15**, 680 (1955).
BRUCE, J., STRONG, J. A.: Maternal hyperparathyroidism and parathyroid deficiency in the child. Quart. J. Med. **24**, 307 (1955).
BUCHS, S.: Angeborener Hypoparathyreoidismus von drei Brüdern infolge von Hyperparathyreoidismus der Mutter. Schweiz. med. Wschr. **91**, 660 (1961).
DAVIS, R. H., FOURMAN, P., SMITH, J. W. G.: Prevalence of parathyroid insufficiency after thyroidectomy. Lancet **1961 II**, 1432.
EIPE, J., JOHNSON, S. A., KIAMKO, R. T., BRONSKY, D.: Hypoparathyroidism following ^{131}I therapy for hyperthyroidism. Arch. intern. Med. **121**, 270 (1968).
FANCONI, A.: Hypoparathyroidismus im Kindesalter. Ergebn. inn. Med. Kinderheilk., N.F. **28**, 54 (1969).
— PRADER, A.: Transient congenital idiopathic hypoparathyroidism. Helv. paediat. Acta **22**, 342 (1967).
FOURMAN, P.: Calcium metabolism and the bone. Oxford: Blackwell Scientific Publication 1960.
FRIDERICHSEN, C.: Tetany in a suckling with latent osteitis fibrosa in the mother. Lancet **1939 I**, 85.
GARDNER, L. I.: Asymptomatic hyperparathyroidism in mother, baby with tetany. Lancet **1961 I**, 173.
KLOTZ, H. P., KANOVITCH, D.: Recherche des signes d'hypoparathyroïdisme incomplet et de tétanie latente dans les affections thyroïdiennes traitées par thyroïdectomie. Marseille-méd. **104**, No 1, 15 (1967).

MIZRAHI, A., GOLD, A.P.: Neonatal tetany secondary to maternal hyperparathyroidism. J. Amer. med. Ass. **190**, 155 (1964).
TIGHE, W.J.: Temporary hypoparathyroidism following radioactive iodine treatment for thyrotoxicosis. J. clin. Endocr. **12**, 1220 (1952).
TOWNSEND, J.D.: Hypoparathyroidism following radioactive iodine therapy for intractable angina pectoris. Ann. intern. Med. **55**, 662 (1961).
WALTON, R.L.: Neonatal tetany in two siblings: effect of maternal hyperparathyroidism. Pediatrics **13**, 227 (1954).
WIJNBLADH, H.: Postoperative tetany. A study based upon 40 treated cases. Acta endocr. (Kbh.) **10**, 1 (1952).

Symptoms

Tetany

ALAJOUANINE, TH., CONTAMIN, F., CATHALA, H.P.: Le syndrome tétanie. Paris: Baillère éd. 1958.
BAKWIN, H.: Pathogenesis of tetany of the newborn. Amer. J. Dis. Child. **54**, 1211 (1937).
FANCONI, G.: Die Nebenschilddrüsen. In: Handbuch der inneren Medizin, Vol. VII/1. Berlin-Göttingen-Heidelberg: Springer 1955.
FORBES, G.B.: Clinical features of idiopathic hypoparathyroidism in children. Ann. N.Y. Acad. Sci. **64**, 432 (1956).
HAAS, H.G.: Tetanie bei Störungen des Calcium- und Magnesiumstoffwechsels. Schweiz. med. Wschr. **95**, 742 (1965).
HADORN, W.: Tetanieprobleme. Helv. med. Acta **13**, 251 (1946).
PRADER, A.: Persönl. Mitteilung.
QUANDT, J., PONSOLD, W.: Nebenschilddrüseninsuffizienz und tetanisches Syndrom. Jena: G. Fischer 1959.
STUCKI, P.: Parathyreoprive Tetanie. Praxis **17**, 1 (1949).

Neurologic Changes

ALAJOUANINE, TH., CONTAMIN, F.: Crises à type d'épilepsie tonique sous corticale avec dépôts calcaires intracérébraux et insuffisance parathyroïdienne. Ann. Méd. **55**, 321 (1954).
BEREZIN, S.W., STEIN, J.D.: Idiopathic hypoparathyroidism. Case simulating epilepsy and brain tumor. J. Pediat. **33**, 346 (1948).
COLARIZI, A., REZZA, E.: Tetania cronica da ipoparatiroidismo idiopatico con manifestazioni epilettiche. Acta paediat. lat. (Parma) **9**, 1 (1956).
CONTAMIN, F., NICOLLE, M.H.: Les encéphalopathies hypocalcémiques. I. Aspects symptomatiques. II. Aspects pronostiques et thérapeutiques. Problèmes pratiques et théoriques. Presse méd. **72**, 2917, 3029 (1964).
GOTTA, H., ODORIZ, J.B.: The electroencephalogram in hypoparathyroidism with tetany and epilepsy. J. clin. Endocr. **8**, 674 (1948).
GREENE, J.A., SWANSON, L.W.: Psychosis in hypoparathyroidism with report of five cases. Ann. intern. Med. **14**, 1233 (1941).
HANSTED, C., BRANDT, S.: Electroencephalographic changes in siblings with hypocalcemia due to hypoparathyroidism. Electroenceph. clin. Neurophysiol. **5**, 101 (1953).
HOSKINS, R.G.: The parathyroids and psychosis. J. clin. Endocr. **2**, 611 (1942).
MACINTYRE, I.: Magnesium metabolism. Advanc. intern. Med. **13**, 143 (1967).
MORTELL, E.J.: Idiopathic hypoparathyroidism and mental deterioration. J. clin. Endocr. **6**, 266 (1946).
ODORIZ, J.B., DEL CASTILLO, E.B., MAUPEDI, J.F., DE LA BALZE, F.A.: Parathyroid insufficiency and the human electroencephalogram. J. clin. Endocr. **4**, 493 (1944).
ROBINSON, K.C., KALLBERG, M.H., CROWLEY, M.F.: Idiopathic hypoparathyroidism presenting as dementia. Brit. med. J. **1954II**, 1203.
SCARLETT, E.P., HOUGHTLING, W.J.: Psychosis in hypoparathyroidism. Canad. med. Ass. J. **50**, 351 (1944).
SCHAAF, M., PAYNE, C.A.: Dystonic reactions to prochlorperazin in hypoparathyroidism. New Engl. J. Med. **275**, 991 (1966).
SIMPSON, J.A.: The neurologic manifestations of idiopathic hypoparathyroidism. Brain **75**, 76 (1952).
WINER, N.J.: Hypoparathyroidism of probable encephalopathic origin. J. clin. Endocr. **5**, 86 (1945).
YIM, B.J.B., JAENIKE, J.R.: Idiopathic hypoparathyroidism presenting as a psychosis and complicated by chlorpromazine jaundice. Amer. J. Med. **25**, 478 (1958).

Cerebral Calcification

BIELSCHOWSKY, M.: Kalkkonkremente im Zentralnervensystem. In: Handbuch der Neurologie (BUMKE, O., FOERSTER, O., eds.), Vol. I., p. 199. Berlin: Springer 1935.
CAMBIER, J.: La maladie de Fahr: calcification intracérébrale non artério-scléreuse et ses rapports avec la tétanie. Presse méd. **60**, 765 (1952).
CAMP, J.D.: Symmetrical calcification of the cerebral basal ganglia. Its roentgenologic significance in the diagnosis of parathyroid insufficiency. Radiology **49**, 568 (1947).
EATON, L.MCK., HAINES, S.F.: Parathyroid insufficiency with symmetrical cerebral calcification. Report of three cases in one of which the patient was treated with dihydrotachysterol. J. Amer. med. Ass. **113**, 749 (1939).
LOTMAR, P.: Histopathologische Befunde in Gehirnen von endemischem Kretinismus, Thyreoaplasie und Cachexia thyreopriva. Z. ges. Neurol. Psychiat. **146**, 1 (1933).
MEDILL, E.V.: Bilateral symmetrical calcifications of basal ganglia associated with parathyroid insufficiency. Brit. J. Radiol. **24**, 685 (1951).
OSTERTAG, B.: Die an bestimmte Lokalisation gebundenen Konkremente des Zentralnervensystems und ihre Beziehung zur Verkalkung intracerebraler Gefäße bei gewissen endokrinen Erkrankungen. Virchows Arch. path. Anat. **275**, 828 (1930).
PALUBINSKAS, A.J., DAVIES, J.: Calcification of the basal ganglia of the brain. Amer. J. Roentgenol. **82**, 806 (1959).
PICK, A.: Weiterer Beitrag zur Pathologie der Tetanie nebst einer Bemerkung zur Chemie verkalkter Hirngefäße. Zbl. ges. Neurol. Psychiat. **22**, 754 (1903).
SCHAAF, M., PAYNE, C.A.: Dystonic reactions to prochlorperazin in hypoparathyroidism. New Engl. J. Med. **275**, 991 (1966).
SCHNABEL: Zur Ätiologie und Histogenese der Verkalkung der kleinen Hirngefäße. Zbl. allg. Path. path. Anat. **33**, 226 (1923).
SIGLIN, J., EATON, L., CAMP, J., HAINES, S.: Symmetric calcification which followed postoperative parathyroid insufficiency. J. clin. Endocr. **7**, 433 (1947).
WEINMANN, W.: Zur Kenntnis der Verkalkung intracerebraler Gefäße. Z. ges. Neurol. Psychiat. **76**, 533 (1922).

Cataract and Other Ocular Changes

BARR, D.P., MACBRYDE, C.M., SANDERS, T.E.: Tetany with pressure I.C. elevated and papilledema. Results of dihydrotachysterol. Trans. Ass. Amer. Phycns. **53**, 227 (1938).
CLARK, J.H.: The effect of parathyroid hormone on the permeability of the lens capsule to calcium. Amer. J. Physiol. **126**, 136 (1939).
COLLINS-WILLIAMS, C.: Idiopathic hypoparathyroidism with papilledema in a boy of 6 years of age: report of a case associated with moniliasis and celiac syndrome and brief review of the literature. Pediatrics **5**, 998 (1950).

POHJOLA, S.: Ocular manifestations of idiopathic hypoparathyroidism. Acta ophthal. (Kbh.) **40**, 255 (1962).
RHYNE, J.L., CARRIKER, F.R.: Idiopathic hypoparathyroidism and bilateral congenital glaucoma in the neonatal period. Pediatrics **18**, 448 (1956).

Trophic Changes in the Skin, Nails, and Hair; Moniliasis (cf. Idiopathic Hypocalcemia, Autoimmune Antibodies)

COLLINS-WILLIAMS, C.: Idiopathic hypoparathyroidism with papilledema in a boy of 6 years of age: report of a case associated with moniliasis and celiac syndrome and brief review of the literature. Pediatrics **5**, 998 (1950).
DENT, C.E., GARRETTS, M.: Skin changes in hypocalcaemia. Lancet **1960 I**, 142.
LEARNER, N.: Ectodermal disorders in chronic hypoparathyroidism. J. clin. Endocr. **3**, 261 (1943).
MCLEAN, F.C.: Chronic idiopathic hypoparathyroidism associated with moniliasis. Arch. Dis. Childh. **29**, 419 (1954).
STICKLER, G.B., PEYLA, T.L., DOWER, J.C., LOGAN, G.B.: Moniliasis, steatorrhea, diabetes mellitus, cirrhosis, gallstones and hypoparathyroidism in a 10-year-old boy. Clin. Pediat. (Phila.) **4**, 276 (1965).
SUTPHIN, A., ALBRIGHT, F., MCCUNE, D.J.: Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis. J. clin. Endocr. **3**, 625 (1943).

Pathology of the Teeth

ALBRIGHT, F., STROCK, M.S.: The association of acalcification of dentine with hypoparathyroidism in rats and the cure of same with parathormone with some correlated observations in man. J. clin. Invest. **12**, 974 (1933).
ERDHEIM, J.: Zur Kenntnis der parathyreopriven Dentinveränderungen. Frankfurt. Z. Path. **7**, 238 (1911).
HANSTED, CH., HOLST, G.: Changes in dental tissue in hypoparathyroidism. Acta odont. scand. **10**, 71 (1952).
LOVESTEDT, S.A.: The dental picture of spontaneous parathyroid insufficiency. Oral Surg. **3**, 396 (1950).
NALLY, J.N., COURVOISIER, B.: Etude des anomalies dentaires dans les syndromes d'insuffisance parathyroïdienne. Rev. mens. Suisse odont. **63**, 764 (1953).
SCHOUR, I., MASSLER, M.: Studies in tooth development: the growth pattern of human teeth. J. Amer. dent. Ass. **27**, 11 (1940).

Pathology of the Skeleton and Joints

ALBRIGHT, F.: Hypoparathyroidism as a cause of osteomalacia. J. clin. Endocr. **16**, 419 (1956).
BELL, N.H., GERARD, E.S., BARTTER, F.C.: Pseudohypoparathyroidism with osteitis fibrosa cystica and impaired absorption of calcium. J. clin. Endocr. **23**, 759 (1963).
BÜCHER, B.: Osteoarthropathia parathyreopriva sclerotica. Röntgenpraxis **17**, 252 (1948).
COURVOISIER, B., BERTHOUD, E., ZAHND, G., GRANDJEAN, A.: Données actuelles sur la physiologie et la pathologie clinique des parathyroïdes. Helv. med. Acta **26**, Suppl. 38 au no. 5 (1959).
FANCONI, A., HEINRICH, H.G., PRADER, A.: Klinischer und biochemischer Hypoparathyreoidismus mit radiologischem Hyperparathyreoidismus. Helv. paediat. Acta **19**, 181 (1964).
IBBERSTSON, H.K., FRASER, R.: Idiopathic hypoparathyroidism with thin bones. Proc. roy. Soc. Med. **51**, 202 (1958).
KOLB, F.O., STEINBACH, H.L.: Pseudohypoparathyroidism with secondary hyperparathyroidism and osteitis fibrosa. J. clin. Endocr. **22**, 59 (1962).
MARTIN, E.: Pers. comm. (1966).
MOLINOFF, S.: Les modifications du squelette dans l'hypoparathyroïdisme. Thèse Genève. Médecine et Hygiène, éd. Genève (1957).
REYNOLDS, T.B., JACOBSON, G., EDMONDSON, H.A., MARTIN, H.E., NELSON, C.H.: Pseudohypoparathyroidism: report of a case showing bony demineralization. J. clin. Endocr. **52**, 560 (1952).
SHULMAN, J.L., RATNER, H.: Idiopathic hypoparathyroidism with bony demineralization and cardiac decompensation. Pediatrics **16**, 848 (1955).

Idiopathic Hypoparathyroidism and Pseudohypoparathyroidism

Definition, Clinical Picture, Hereditary Factors, Pathogenesis

ALBRIGHT, F., BURNETT, CH.H., SMITH, P.H., PARSON, W.: Pseudohypoparathyroidism — an example of "Seabright-Bantam Syndrome". Endocrinology **30**, 922 (1942).
— FORBES, A., HENNEMAN, P.: Pseudo-pseudohypoparathyroidism. Trans. Ass. Amer. Phycns **65**, 337 (1952).
ALEXANDER, S.B., TUCKER, H.: Pseudohypoparathyroidism. Report of a case with late manifestation. J. clin. Endocr. **9**, 863 (1949).
ALIAPOULIOS, M.A., VOELKEL, E.F., MUNSON, P.L.: Assay of human thyroid glands for thyrocalcitonin activity. J. clin. Endocr. **26**, 897 (1966).
ARDEN, F.: Idiopathic hypoparathyroidism. Case report. Med. J. Aust. **40**, 2 (1953).
AURBACH, G.D., POTTS, J.T., CHASE, L.R., MELSON, G.L.: Polypeptides hormones and calcium metabolism. Ann. intern. Med. **70**, 1243 (1969).
BARTTER, F.C.: Pseudohypoparathyroidism and pseudopseudohypoparathyroidism. In: STANBURY, J.B., WYNGAARDEN, J.B., FREDRICKSON, D.S. (eds.), The metabolic basis of inherited disease, 2nd ed., p. 1024, New York: McGraw-Hill Book Co., 1966.
BELL, N.H., GERARD, E.S., BARTTER, F.C.: Pseudohypoparathyroidism with osteitis fibrosa cystica and impaired absorption of calcium. J. clin. Endocr. **23**, 759 (1963).
BERGIN, J.D., KEELING, J.: Idiopathic hypoparathyroidism. N. Z. med. J. **62**, 401 (1963).
BERGSTRAND, C.G., EKENGREN, K., FIFIPSSON, R., HUGGERT, A.: Pseudohypoparathyroidism. Familial incidence and comparison with idiopathic hypoparathyroidism. Acta endocr. (Kbh.) **29**, 201 (1958).
BRONSKY, D.: Hyperparathyroidism with Albright's osteodystrophy: Case report and a proposed new classification of parathyroid disease. J. clin. Endocr. **31**, 271 (1970).
— KUSHNER, D.S., SNAPPER, I.: Idiopathic hypoparathyroidism and pseudohypoparathyroidism. Case reports and review of literature. Medicine (Baltimore) **37**, 317 (1958).
BUCHS, S.: Familiärer Hypoparathyreoidismus. Ann. paediat. (Basel) **184**, 364 (1955); **188**, 124 (1957).
CATHALA, J., POLONOVSKI, CL., BARRE, CL.: L'hypoparathyroïdie chronique primitive de l'enfant. Presse méd. **66**, 1925 (1958).
CHASE, L.R., AURBACH, G.D.: Parathyroid function and the renal excretion of 3',5'-adenylic acid. Proc. nat. Acad. Sci. (Wash.) **58**, 518 (1967).
— — Renal adenyl cyclase; anatomically separate sites for parathyroid hormone and vasopressin. Science **159**, 545 (1968).
— FEDAK, S.A., AURBACH, G.D.: Activation of skeletal adenyl cyclase by parathyroid hormone in vitro. J. Endocr. **84**, 761 (1969).
— MELSON, G.L., AURBACH, G.D.: Pseudohypoparathyroidism: Defective excretion of 3',5'-AMP in response to parathyroid hormone. J. clin. Invest. **48**, 1832 (1969).

COHEN, M.L., DONNELL, G.N.: Pseudohypoparathyroidism with hypothyroidism. A case report and review of literature. J. Pediat. **56**, 369 (1960).

COURVOISIER, B., BERTHOUD, E., ZAHND, G., GRANDJEAN, A.: Données actuelles sur la physiologie et la pathologie clinique des parathyroïdes. Helv. med. Acta **26**, Suppl. 38 au no. 5 (1959).

ELRICK, H., ALBRIGHT, F., BARTLES, F., FORBES, A.P., REEVES, J.D.: Further studies on pseudohypoparathyroidism. Report of four new cases. Acta endocr. (Kbh.) **5**, 199 (1950).

FEDAK, S.A., AURBACH, G.D.: Activation of skeletal adenyl cyclase of parathyroid hormone. Endocrinology **84**, 761 (1969).

FISCHER, J.A., BINSWANGER, U., FREY-WETTSTEIN, M.: "Hypoparathyroidism", hyperostosis, normophosphatemia and thyrocalcitonin. Acta endocr. (Kbh.) **55**, 562 (1967).

FOSTER, G.V., DOYLE, F.H., BORDIER, P., MATRAJT, H.: Effect of thyrocalcitonin on bone. Lancet **1966 II**, 1428.

FRAME, B., HANSON, CH.A., FROST, H., BLOCK, M., ARNSTEIN, A.R.: Renal resistance to parathyroid hormone with osteitis fibrosa. Ambr. J. Med. **52**, 311 (1972).

GOLDMAN, R., REYNOLDS, J.L., CUMMINGS, H.R., BASSETT, S.H.: Familial hypoparathyroidism: report of a case. J. Amer. med. Ass. **150**, 1104 (1952).

GSELL, O.: Chronische idiopathische Tetanie (mit Psoriasis) (hypoparathyreoider Kretinismus). Dtsch. med. Wschr. **35**, 1117 (1950).

HAGEN, G.A.: Renal excretion of calcium and phosphorus in pseudohypoparathyroidism. J. clin. Endocr. **24**, 1244 (1964).

KOLB, F.O., STEINBACH, H.L.: Pseudohypoparathyroidism with secondary hyperparathyroidism and osteitis fibrosa. J. clin. Endocr. **22**, 59 (1962).

LEE, J.B., TASHJIAN, A.H., JR., STREETO, J.M., FRANTZ, A.G.: Familial pseudohypoparathyroidism: Role of parathyroid hormone and thyro-calcitonin. New Engl. J. Med. **229**, 1179 (1968).

MCDONALD, K.M.: Responsiveness of bone to parathyroid extract in siblings with pseudohypoparathyroidism. Metabolism **21**, 521 (1972).

MAMOU, H., SEE, P.: Pseudo-hypoparathyroïdisme, un cas familial chez deux soeurs jumelles. Sem. Hôp. Paris **31**, 3513 (1955).

MACKLER, H., FOUTS, J.R., BIRSNER, J.W.: Familial pseudohypoparathyroidism: report of 2 cases. Calif. Med. **77**, 5 (1952).

MANN, J.B., ALTERMAN, S., HILLS, A.G.: Albright's hereditary osteodystrophy comprising pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism. Ann. intern. Med. **56**, 315 (1962).

MARTIN, E.: Pers. comm. (1964).

— BOURDILLON, J.: Un cas de tétanie idiopathique chronique. Echec thérapeutique de la greffe d'un adénome parathyroïdien. Rev. méd. Suisse rom. **14**, 1166 (1940).

MOWBRAY, R.R., DE, SMITH, SH.L., SYMONDS, W.J.: Hypoparathyroidism and pseudohypoparathyroidism. Brit. med. J. **1954**, 903.

NAGENT DE DEUXCHAISNES, C., FANCONI, A., ALBERTO, P., RUDLER, J.C., MACH, R.S.: Phéochromocytomes extra-surrénaliens multiples avec «dystrophie d'Albright» et hémangiomes cutanés. Schweiz. med. Wschr. **90**, 886 (1960).

— ISAAC, G., JACQUET, A., HOET, J.J.: Etude clinique et physio-pathogénique de la «dystrophie d'Albright» (pseudo-pseudohypoparathyroïdisme) et des syndromes voisins. A propos de trois nouveaux cas, dont deux familiaux. Rev. franç. Étud. clin. biol. **5**, 153 (1960).

NICHOLS, F., HOLDSWORTH, D., REINFRANK, R.: Familial hypocalcemia, latent tetany and calcification of the basal ganglia. Amer. J. med. **30**, 518 (1961).

PECHET, M.M., RASMUSSEN, H., CARROLL, E.Z., CRAMER, I.: Studies with purified bovine parathormone. Effects in normal hypoparathyroid and pseudohypoparathyroid subjects. J. clin. Invest. **40**, 1070 (1961).

PEDEN, V.H.: True idiopathic hypoparathyroidism as a sex-linked recessive trait. Amer. J. hum. Genet. **12**, 323 (1960).

REYNOLDS, T.B., JACOBSON, G., EDMONDSON, H.A., MARTIN, H.E., NELSON, C.H.: Pseudohypoparathyroidism: report of a case showing bony demineralization. J. clin. Endocr. **52**, 560 (1952).

ROBINSON, P.K., CARMICHAEL, E.A., CUMINGS, J.N.: Idiopathic hypoparathyroidism. A study of three cases. Quart. J. Med. **23**, 383 (1954).

SALVESEN, H.A., BOE, J.: Idiopathic hypoparathyroidism. Acta endocr. (Kbh.) **14**, 214 (1953).

SCHÜPBACH, A., COURVOISIER, B.: Existe-t-il un pseudo-hypoparathyroïdisme? Schweiz. med. Wschr. **79**, 887 (1949).

STEINBACH, H.L., YOUNG, D.A.: The roentgen appearence of pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism. Amer. J. Roetgenol. **97**, 49 (1966).

STEINBERG, H., WALDRON, B.R.: Idiopathic hypoparathyroidism; an analysis of fifty-two cases including the report of a new case. Medicine (Baltimore) **31**, 133 (1952).

STROM, L., WINBERG, J.: Idiopathic hypoparathyroidism. Acta paediat. (Uppsala) **43**, 574 (1954).

SUH, S.M., KOOH, S.W., CHAN, A.M., FRASER, D., TASHJIAN, A.R.: Pseudohypoparathyroidism: No improvement following total thyroidectomy. J. clin. Endocr. **29**, 429 (1969).

TASHJIAN, A.H., JR., FRANTZ, A.G., LEE, J.B.: Pseudohypoparathyroidism. Assay of parathyroid hormone and thyrocalcitonin. Proc. nat. Acad. Sci. (Wash.) **56**, 1138 (1966).

WALKER, D.G.: Counteraction of parathyroid therapy in osteopetrotic mice as revealed in the plasma calcium level and ability to incorporate ^{3}H-proline into bone. Endocrinology **79**, 836 (1966).

WINNACKER, J.L., BECKER, K.L., MOORE, C.F.: Pseudohypoparathyroidism and selective deficiency of thyrotropin: an interesting association. Metabolism **16**, 644 (1967).

ZAMPA, G.A., ZUCCHELLI, P.C.: Pseudohypoparathyroidism and bone demineralization: case report and metabolic studies. J. clin. Endocr. **25**, 1616 (1965).

ZELTNER, L.: Les hypocalcémies «idiopathiques». Etude anatomopathologique et génétique. Praxis **58**, 497 (1969).

ZISMAN, E., LOTZ, M., JENKINS, M.E., BARTTER, C.: Studies in pseudohypoparathyroidism. Two new cases with a probably selective deficiency of thyrotropin. Amer. J. Med. **46**, 464 (1969).

Differential Diagnosis

ARNAUD, C.D., TSAO, H.S., LITTLEDIKE, T.: Radioimmunoassay of human parathyroid hormone in serum. J. clin. Invest. **50**, 21 (1971).

FISCHER, J.A., BINSWANGER, U., FANCONI, A., ILLIG, R., BAERLOCHER, K., PRADER, A.: Serum parathyroid hormone concentrations in vitamin D deficiency rickets of infancy: Effects of intravenous calcium and vitamin D. Submitted for publication (1973).

REISS, E., CANTERBURY, J.M.: A radioimmunoassay for parathyroid hormone in man. Proc. Soc. exp. Biol. (N.Y.) **128**, 501 (1968).

Idiopathic Hypocalcemia

ALBRIGHT, F., BURNETT, CH.H., SMITH, P.H., PARSON, W.: Pseudohypoparathyroidism — an example of "Seabright-Bantam syndrome". Endocrinology **30**, 922 (1942).

BÖTTIGER, E., WERNSTEDT, W.: Beiträge zur Kenntnis der spasmophilen Diathese. IV. Mitteilung. Tödlich verlaufender Fall von Spasmophilie bei einem Brustkinde mit Anomalien der Thymus und der Parathyreoideae. Acta paediat. (Uppsala) **6**, 373 (1927).

CHU, H.Y., CHANG, C., YIN, W.: Idiopathic hypoparathyroidism; report of 14 cases with one autopsy record. Chin. med. J. **83**, 723 (1964).

DRAKE, T.G., ALBRIGHT, F., BAUER, W., CASTLEMAN, B.: Chronic idiopathic hypoparathyroidism. Report of 6 cases with autopsy finding in one. Ann. intern. Med. **12**, 1751 (1939).

FRAME, B., FRUCHTMAN, M., SMITH, R.W.: Chronic hypocalcemia in a patient with parathyroid clear-cell hyperplasia. New Engl. J. Med. **267**, 1112 (1962).

GILMOUR, J.R.: Some developmental abnormalities of the thymus and parathyroids. J. Path. Bact. **52**, 213 (1941).

RÖSSLE, R.: Über den angeborenen Mangel der Epithelkörperchen. Schweiz. med. Wschr. **68**, 848 (1938).

ZELTNER, L.: Les hypocalcémies «idiopathiques». Etude anatomopathologique et génétique. Praxis **58**, 497 (1969).

Autoimmune Antibodies, Addison's Disease, Pernicious Anemia (cf. Hypoparathyroidism, Trophic Changes)

BLIZZARD, R.M., CHEE, D., DAVIS, W.: The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism. Clin. exp. Immunol. **1/2**, 119 (1966).

BRAUN, W.: Das Syndrom Hypocortizismus (Morbus Addison) mit Hypoparathyreoidismus im Kindesalter. Kinderärztl. Prax. **32**, 207 (1964).

BROCKHOUSE, J.P.: Hypoparathyroidism with recurrent hyperthyroidism: a case report. J. Kans. med. Soc. **65**, 527 (1964).

COEN, G., MAZZUOLI, G.F.: Idiopathic hypoparathyroidism associated with adrenal insufficiency—report of a case with a study of calcium metabolism. Folia endocr. (Pisa) **18**, 136 (1965).

CRAIG, J.M., SCHIFF, L.H., BOONE, J.E.: Chronic moniliasis associated with Addison's disease. Amer. J. Dis. Child. **89**, 669 (1955).

DAHL, J.R., MCFADDEN, S.D., EISENBERG, E.: Idiopathic hypoparathyroidism associated with hyperthyroidism. Ann. intern. Med. **57**, 635 (1962).

DI GEORGE, A., PASCHKIS, K.: The syndrome of Addison's disease, hypoparathyroidism and superficial moniliasis. Amer. J. Dis. Child. **94**, 476 (1957).

DUBIN, A.: Hyperuricemia in hypoparathyroidism. Metabolism **5**, 703 (1956).

GASS, J.D.: The syndrome of Keratoconjonctivitis, superficial moniliasis, idiopathic hypoparathyroidism and Addison's disease. Amer. J. Ophtal. **54**, 660 (1962).

HETZEL, B.S., ROBSEN, H.N.: The syndrome of hypoparathyroidism, Addison's disease and moniliasis. Aust. Ann. Med. **7**, 27 (1958).

HIEKKALA, H.: Idiopathic hypoparathyroidism, adrenal insufficiency and moniliasis in children. Ann. Paediat. Fenn. **10**, 213 (1964).

HUNG, W., MIGEON, C.J., PARROTT, R.H.: A possible autoimmune basis for Addison's disease in three siblings, one with idiopathic hypoparathyroidism, pernicious anemia and superficial moniliasis. New Engl. J. Med. **269**, 658 (1963).

HURWITZ, L.J.: Spontaneous hypoparathyreoidism with megaloblastic anaemia. Lancet **1956**, 234.

KENNY, F.M., HOLLDAY, M.A.: Hypoparathyroidism, moniliasis, Addison's and Hashimoto's diseases: hypercalcemia treated with intravenously administered sodium sulfate. New Engl. J. Med. **271**, 708 (1964).

LEIFER, E., HOLLANDER, W.: Idiopathic hypoparathyroidism and chronic adrenal insufficiency: a case report. J. clin. Endocr. **13**, 1264 (1953).

LEONARD, M.F.: Chronic idiopathic hypoparathyroidism with superimposed Addison's disease in a child. J. clin. Endocr. **6**, 493 (1946).

MALLOY, B.M., WOODRUFF, C.W.: Addison's disease in three six-year-old boys. Amer. J. Dis. Child. **95**, 364 (1958).

MCMAHON, F.G., COOKSON, D.U., KABLER, J.D., INHORN, S.L.: Idiopathic hypoparathyroidism and idiopathic adrenal cortical insufficiency occurring with cystic fibrosis of the pancreas. Ann. intern. Med. **51**, 371 (1959).

MORSE, W.I., COCHRANE, W.A., LANDRIGAN, P.L.: Familial hypoparathyroidism with pernicious anemia, steatorrhea and adrenocortical insufficiency. New Engl. J. Med. **264**, 1021 (1961).

PERLMUTTER, M., ELLISON, R.R., NORSA, L., KANTROWITZ, A.R.: Idiopathic hypoparathyroidism and Addison's disease. Amer. J. Med. **21**, 634 (1956).

QUINTO, M.G., LEIKIN, S.L., HUNG, W.: Pernicious anemia in a young girl associated with idiopathic hypoparathyroidism familial Addison's disease and moniliasis. J. Pediat. **64**, 241 (1964).

REISNER, D.J., ELLSWORTH, R.M.: Coexistent idiopathic hypoparathyroidism and pernicious anemia in a young girl: case report. Ann. intern. Med. **43**, 1116 (1955).

RUPP, J.J., PASCHKIS, K.E.: Panhypopituitarism and hypocalcemic tetany in male: case presentation. Ann. intern. Med. **39**, 1103 (1953).

SUTPHIN, A., ALBRIGHT, F., MCCUNE, D.J.: Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis. J. clin. Endocr. **3**, 625 (1943).

SZCZEPANSKA, H., SAPIECHA, J.: Addison's disease with hypofunction of the parathyroid gland. Arch. Dis. Childh. **34**, 498 (1959).

WAGNER, R.: The syndrome of chronic hypoparathyroidism, Addison's disease and superficial moniliasis. Exp. Med. Surg. **18**, 157 (1960).

WHITAKER, J., LANDING, B.H., ESSELBORN, V.M., WILLIAMS, R.R.: The syndrome of familial juvenile hypoadrenocorticism, hypoparathyroidism and superficial moniliasis. J. clin. Endocr. **16**, 1374 (1956).

Treatment of Hypoparathyroidism and Idiopathic Hypocalcemia

BEIDLEMAN, B.: Treatment of chronic hypoparathyroidism with probenecid. Metabolism **7**, 690 (1958).

DESPOPOULOS, A.: Probenecid in hypoparathyroidism: absence of phosphaturic response. J. clin. Endocr. **18**, 769 (1958).

GRAHAM, W.P., GORDAN, G.S., LOKEN, H.F., BLUM, A., HALDEN, A.: Effect of pregnancy and of the menstrual cycle on hypoparathyroidism. J. clin. Endocr. **24**, 512 (1964).

HARRISON, H.E., LIFSCHITZ, F., BLIZZARD, R.M.: Comparison between crystalline dihydrotachysterol and calciferol in patients requiring pharmacologic vitamin D therapy. New Engl. J. Med. **276**, 894 (1967).

IRELAND, A.W., CLUBB, J.S., NEALE, F.C., POSEN, S., REEVE, T.S.: The calciferol requirements of patients with surgical hypoparathyroidism. Ann. intern. Med. **69**, 81 (1968).

MELICK, R.A., GILL, J.R., BERSON, S.A., YALOW, R.S., BARTTER, F.C., POTTS, J.T., AURBACH, G.D.: Antibodies and clinical resistance to parathyroid hormone. New Engl. J. Med. **276**, 144 (1967).

PASCALE, L., DUBIN, A., HOFFMAN, W.S.: Influence of benemid in urinary excretion of phosphate in hypoparathyroidism. Metabolism **3**, 462 (1954).

Primary Hyperparathyroidism

Definition, Pathogenesis, Occurrence, Pathophysiology

ALTENÄHR, E., BARTELHEIMER, H., BAY, V., KUHLENCORDT, F., LOZANO-TONKIN, C., SCHNEIDER, C., SEEMANN, N., SEIFERT, G.: Der autonome und regulative Hyperparathyreoidismus. Stuttgart: Enke 1969.

BAUER, G.C.H., CARLSSON, A., LINDQUIST, B.: A comparative study on the metabolism of Sr 90 and Ca 45. Acta physiol. scand. **35**, 56 (1955).

BÉLANGER, L.F., ROBICHON, J.: Parathormone-induced osteolysis in dogs: a microradiographic and alpha-radiographic survey. J. Bone Jt Surg. A **46**, 1008 (1964).

BERSON, S.A.: Immunochemical heterogeneity of parathyroid hormone in plasma. J. clin. Endocr. **28**, 1037 (1968).

— YALOW, R.S.: Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia and bronchogenic carcinom. Science **154**, 907 (1966).

BLACK, B.M.: The pathology and surgery of the parathyroid glands. In: GREEP, R.O., and R.V. TALMAGE, The parathyroids. Springfield: Ch.C. Thomas 1961.

BOONSTRA, C.E., JACKSON, C.E.: Clinical value of routine serum calcium analysis. J. Lab. clin. Med. **56**, 794 (1960).

BRICKER, N.S., FORREST, J., AVIOLI, L., SLATOPOLSKY, E., KISSANE, J., CHASE, L., LUBOWITZ, H.: Tertiary hyperparathyroidism—does it exist? Amer. J. Med. **52**, 254 (1972).

BUCKLE, R.M.: Hyperparathyroidism in chronic renal failure. Lancet **1970II**, 234.

CARE, A.D., SHERWOOD, L.M., POTTS, J.T., AURBACH, G.D.: Perfusion of the isolated parathyroid gland of the goat and sheep. Nature (Lond.) **209**, 55 (1966).

DENT, C.E., WATSON, L.: Hyperparathyroidism and sarcoidosis. Brit. med. J. **1966I**, 646.

HANNA, S., HARRISON, M., MACINTYRE, I., FRASER, R.: The syndrome of magnesium deficiency in man. Lancet **1960II**, 172.

— NORTH, K.A.K., MACINTYRE, I., FRASER, R.: Magnesium metabolism in parathyroid disease. Brit. med. J. **1961II**, 1253.

HEATON, F.W.: The parathyroid glands and magnesium metabolism in the rat. Clin. Sci. **28**, 543 (1965).

HELLSTRÖM, J.: Primärer Hyperparathyreoidismus. Triangle (En.) **5**, 171 (1961).

JOWSEY, J., RIGGS, B.I., KELLY, P.J.: Mineral metabolism in osteocytes. Proc. Mayo Clin. **39**, 480 (1964).

MACINTYRE, I., BOSS, S., TROUGHTON, V.A.: Parathyroid hormone and magnesium homeostasis. Nature (Lond.) **198**, 1058 (1963).

PALOYAN, E., PALOYAN, D., HARPER, P.V.: The role of glucagon hypersecretion in the relationship of pancreatitis and hyperparathyroidism. Surgery **62**, 167 (1967).

POTTS, J.T., MURRAY, T.M., PEACOCK, M., NIALL, H.D., TREGEAR, G.W., KEUTMANN, H.T., POWELL, D., DEFTOS, L.J.: Parathyroid hormone: sequence, synthesis, immunoassay studies. Amer. J. Med. **50**, 639 (1971).

REISS, E., CANTERBURY, J.M.: Primary hyperparathyroidism. Differentiation of adenoma and hyperplasia and localization of hyperfunctioning glands. New Engl. J. Med. **280**, 1381 (1969).

ROTH, S.I.: Pathology of the parathyroids in hyperparathyroidism. Arch. Path. **73**, 495 (1962).

SUTTON, R.A.: Plasma magnesium concentration in primary hyperparathyroidism. Brit. med. J. **1970I**, 529.

VOSIK, W.M., ANDERSON, C.F., STEFFEES, W.P., JOHNSON, W.J., ARNAUD, C.D., GOLDSMITH, R.S.: Successful medical management of osteitis fibrosa due to "tertiary" hyperthyroidism. Mayo Clin. Proc. **47**, 110 (1972).

WERMER, P.: Endocrine adenomatosis and peptic ulcer in a large kindred. Inherited multiple tumors and mosaic pleiotropism in man. Amer. J. Med. **35**, 205 (1963).

WILSON, R.E.: Diskussionsbemerkung zu PALOYAN et al. Surgery **62**, 172 (1967).

Pathology and Histology

ABUL-HAJ, S.K., CONKLIN, H., HEWITT, W.C.: A functioning lipo-adenoma of the parathyroid gland. Report of a unic case. New Engl. J. Med. **266**, 121 (1962).

ADAMS, P.H., CHALMERS, T.M., PETERS, N., RACK, J.H., TRUSCOTT, B.McN.: Primary chief cell hyperplasia of the parathyroid glands. Ann. intern. Med. **63**, 454 (1965).

ALBERTINI, A.v.: Histologische Geschwulstdiagnostik. Stuttgart: Georg Thieme 1955.

BLACK, B.M.: The pathology and surgery of the parathyroid glands. In: GREEP, R.O., and R.V. TALMAGE, The parathyroids. Springfield: Ch.C. Thomas 1961.

CASTLEMAN, B.: Tumors of parathyroid glands. Armed Forces Institute of Pathology. Washington 1952.

COPE, O., KEYNES, W.M., ROTH, S.I., CASTLEMAN, B.: Primary chief-cell hyperplasia of the parathyroid glands: a new entity in the surgery of hyperparathyroidism. Ann. Surg. **148**, 375 (1958).

CUTLER, R.E., REISS, E., ACKERMAN, L.V.: Familial hyperparathyroidism. A kindred involving 11 cases, with a discussion of primary chief-cell hyperplasia. New Engl. J. Med. **270**, 859 (1964).

ELLIOT, R.L., ARHELGER, R.B.: Fine structure of parathyroid adenomas, with special reference to annulate lammellae and septate desmosomes. Arch. Path. **81**, 200 (1966).

HARRIS, J.C., ROSENBAUM, D., OLINER, L.: Primary hyperparathyroidism with parathyroid hyperplasia and adenoma in a single gland. Amer. J. clin. Path. **45**, 51 (1966).

HELLSTRÖM, J.: Primärer Hyperparathyreoidismus. Triangle (En.) **5**, 171 (1961).

HOLZMANN, K., LANGE, R.: Zur Cytologie der Glandula parathyreoidea des Menschen. Weitere Untersuchungen an Epithelkörperadenomen. Z. Zellforsch. **58**, 759 (1963).

HOWARD, J.E., FOLLIS, R.H., YENDT, E.R., CONNOR, T.B.: Hyperparathyroidism. Case report illustrating spontaneous remission due to necrosis of adenoma, and a study of the incidence of necrosis in parathyroid adenomas. J. clin. Endocr. **13**, 997 (1953).

JOHNSTON, C.C., SCHNUTE, R.B.: A case of primary hyperparathyroidism with spontaneous remission following infarction of the adenoma with development of hypocalcemic tetany. J. clin. Endocr. **21**, 196 (1961).

LANGE, R.: Zur Histologie und Zytologie der Glandula parathyreoidea des Menschen. Licht- und elektronenmikroskopische Untersuchungen an Epithelkörperadenomen. Z. Zellforsch. **53**, 765 (1961).

MELVIN, K.E.W., CASTLEMAN, B.: Case records of the Massachusetts General Hospital (Case 50-1971), Persistent hypercalcemia after removal of a parathyroid tumor. New Engl. J. Med. **285**, 1422 (1971).

OBER, W.B., KAISER, C.A.: Hamartoma of the parathyroid. Cancer (Philad.) **11**, 601 (1958).

RAMSDELL, J.A., BLACK, B.M., WOOLNER, L.B., KEATING, F.R., JR.: Tumors of parathyroid glands found incidentally during surgical procedures. Surg. Gynec. Obstet. **111**, 451 (1960).

ROTH, S.I.: Pathology of the parathyroids in hyperparathyroidism. Arch. Path. **73**, 495 (1962).

— Recent advances in parathyroid gland pathology. Amer. J. Med. **50**, 612 (1971).

— MUNGER, B.L.: The cytology of the adenomatous, atrophic and hypertrophic parathyroid glands of man. Virchows Arch. path. Anat. **335**, 389 (1962).

WERNLY, M., BERDJIS-CHAMSI, CH.: Les parathyroïdes humaines. Contribution à l'étude des hyperplasies et des adénomes. Basel: Benno Schwabe & Co. 1946.

Clinical Picture, Diagnosis, Differential Diagnosis

AITKEN, R.E., KERR, J.L., LLOYD, H.M.: Primary hyperparathyroidism with osteosclerosis and calcification in articular cartilage. Amer. J. Med. **37**, 813 (1964).

ALBRIGHT, F., REIFENSTEIN, E.C.: The parathyroid glands and metabolic bone disease. Baltimore: Williams & Wilkins Co. 1948.

ARNAUD, C.D., TSAO, H.S., LITTLEDIKE, D.: Radioimmunoassay of human parathyroid hormone in serum. J. clin. Invest. (im Druck) (1971).

BARRERAS, R.F., DONALDSON, R.M.: Role of calcium in gastric hypersecretion, parathyroid adenoma and peptic ulcer. New Engl. J. Med. **276**, 1122 (1967).

BAUER, G.C.H., CARLSSON, A., LINDQUIST, B.: A comparative study on the metabolism of Sr 90 and Ca 45. Acta physiol. scand. **35**, 56 (1955).

BERSON, S.A., YALOW, R.S.: Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia and bronchogenic carcinoma. Science **154**, 907 (1966).

BLACK, B.M.: Hyperparathyroidism. Springfield: Ch.C. Thomas 1953.

— The pathology and surgery of the parathyroid glands. In: GREEP, R.O., and R.V. TALMAGE, The parathyroids. Springfield: Ch.C. Thomas 1961.

BLEULER, E.: Endokrinologische Psychiatrie. Stuttgart: Thieme 1954.

BOONSTRA, C.E., JACKSON, C.E.: Clinical value of routine serum calcium analysis. J. Lab. clin. Med. **56**, 794 (1960).

BOYCE, D., JOWSEY, J.: Measurement of osteoid tissue in primary hyperparathyroidism. Proc. Mayo Clin. **41**, 836 (1966).

BUCKLE, R.M.: Hyperparathyroidism in chronic renal failure. Assessment of autonomy by plasma-parathyroid-hormone response to alterations in calcium. Lancet **1970II**, 234.

BYWATERS, E.G.L., DIXON, A.ST.J., SCOTT, J.T.: Joint lesions of hyperparathyroidism. Ann. rheum. Dis. **22**, 171 (1963).

COLLIP, J.B.: The parathyroid glands. Medicine (Baltimore) **5**, 1 (1926).

CONNOR, T.B., THOMAS, W.C., JR., HOWARD, J.E.: The etiology of hypercalcemia associated with lung carcinoma. J. clin. Invest. **35**, 697 (1956).

COPE, O., CULVER, P.I., MIXTER, C.G., NARDI, G.L.: Pancreatitis, a diagnostic clue to hyperparathyroidism. Ann. Surg. **145**, 857 (1957).

DENT, C.E.: Some problems of hyperparathyroidism. Brit. med. J. **1962II**, 1419, 1495.

DUARTE, C.G., WINNACKER, J.L., BECKER, K.L., PACE, A.: Thiazide-induced hypercalcemia. New Engl. J. Med. **284**, 828 (1971).

EDWARDS, G.A., DAUM, S.M.: Increased spinal fluid protein in hyperparathyroidism and other hypercalcemic states. Arch. intern. Med. **104**, 29 (1959).

Editorial: Factors in the relation of parathyroid and gastric function. New Engl. J. Med. **276**, 1154 (1967).

ENGFELDT, B., GARDELL, S., HELLSTRÖM, I., IVEMARK, B., RHODIN, I., STRANDH, J.: Effect of experimentally induced hyperparathyroidism on renal function and structure. Acta endocr. (Kbh.) **29**, 15 (1958).

FANCONI, A., MIETH, D.: Primärer Hyperparathyreoidismus bei einem 12jährigen Knaben. Helv. paediat. Acta **22**, 160 (1967).

FRAME, B., HAUBRICH, W.S.: Peptic ulcer and hyperparathyroidism: survey of 300 ulcer patients. Arch. intern. Med. **105**, 536 (1960).

GERSTER, J.C., GAUTHIER, G.: Polyneuropathy in a case of primary hyperparathyroidism: Disappearance of the neurological picture after surgical correction of the hyperparathyroidism. Helvet. med. Acta **35**, 296 (1969/70).

GORDAN, G.S.: In: R. ESCAMILLA, Laboratory tests in diagnosis and investigation of endocrine functions. Oxford: Blackwell Scientific Publications 1962.

HAAS, H.G.: Der heutige Stand der Diagnose des Hyperparathyreoidismus. Praxis **52**, 140 (1963).

— Knochenstoffwechsel und Parathyreoidea-Erkrankungen. Stuttgart: Georg Thieme 1966.

HARRISON, M., FRASER, R.: Hyperparathyroidism. Proc. roy. Soc. Med. **52**, 255 (1959).

— HARDEN, R.MCD., ALEXANDER, W.D.: Some effects of parathyroid hormone in thyrotoxicosis. J. clin. Endocr. **24**, 214 (1964).

HARTENSTEIN, H., GARDNER, L.I.: Tetany of the newborn associated with maternal parathyroid adenoma. New Engl. J. Med. **274**, 266 (1966).

HELLSTRÖM, J.: Primärer Hyperparathyreoidismus. Triangle (En.) **5**, 171 (1961).

— The treatment of hyperparathyroidism. Urol. int. (Basel) **19**, 124 (1965).

HODGKINSON, A.: Biochemical aspects of primary hyperparathyroidism. Clin. Sci. **25**, 231 (1963).

HORN, H.D., NIETH, H., GROTE, G.: Untersuchungen über die renale Phosphatexkretion bei Kranken mit verschiedenen Nephropathien unter besonderer Berücksichtigung von Kranken mit primärem Hyperparathyreoidismus. Klin. Wschr. **42**, 991 (1964).

HOWARD, J.E.: Clinical and laboratory research concerning mechanism of formation and control of calculous disease by the kidney. J. Urol. (Baltimore) **72**, 999 (1954).

— FOLLIS, R.H., YENDT, E.R., CONNOR, T.B.: Hyperparathyroidism. Case report illustrating spontaneous remission due to necrosis of adenoma, and a study of the incidence of necrosis in parathyroid adenomas. J. clin. Endocr. **13**, 997 (1953).

JOWSEY, J.: Quantitative microradiography. Amer. J. Med. **40**, 485 (1966).

— Measurements of osteoid tissue in primary hyperparathyreoidism. Proc. Mayo Clin. **41**, 836 (1966).

— RIGGS, B.L., KELLY, P.J.: Mineral metabolism in osteocytes. Proc. Mayo Clin. **39**, 480 (1964).

KARPATI, G., FRAME, B.: Neuropsychiatric disorders in primary hyperparathyroidism. Arch. Neurol. (Chic.) **10**, 387 (1964).

KEATING, F.R.: Personal communication 1965.

KEATING, F.R., JR.: Diagnosis of primary hyperparathyroidism. J. Amer. med. Ass. **178**, 547 (1961).

KEYNES, M.: The clinical diagnosis of primary hyperparathyroidism. Geriatrics **20**, 65 (1965).

KIND, H.: Psychische Störungen bei Hyperparathyreoidismus. Arch. Psychiat. Nervenkr. **200**, 1 (1959).

KRAWITT, E.L., BLOOMER, H.A.: Increased cerebrospinal fluid protein secondary to hypercalcemia of the milk-alkali-syndrome. New Engl. J. Med. **273**, 154 (1965).

KYLE, L.H.: Cited by MIXTER (1962).

LICHTWITZ, A., PARLIER, R., SÈZE, S. DE, HIOCO, D., MIRAVET, L.: L'effet hypocalciurique des sulfamides diurétiques. Sem. Hôp. Paris **37**, 2350 (1961).

— SÈZE, S. DE, HIOCO, D., BORDIER, P.H., MAZABRAUD: Ostéomalacie par hyperostéoblastose et formes ostéomalaciques des l'hyperparathyroïdie. Presse méd. **64**, 2033 (1956); **65**, 45 (1957).

LIÈVRE, J.-A.: La maladie osseuse de Paget. Praxis **56**, 1252 (1967).

— FISCHGOLD, H.: Crâne et face dans la maladie de Paget. Paris: Masson & Cie. 1959.

LOMNITZ, E., SEPULVEDA, L., STEVENSON, C., BARZELATTO, J.: Primary hyperparathyroidism simulating rickets. J. clin. Endocr. **26**, 309 (1966).

LORME, A. DE: Hypercalcämie bei M. Addison. Dissertation, Zürich, 1965.

LUDWIG, G.D., CHAYKIN, L.: Pancreatitis associated with primary hyperparathyroidism. Med. Clin. N. Amer. **50**, 1403 (1966).

LYNCH, H.T., LEMON, H.M., HENN, M.J., ELLINGSON, R.J., GRISSOM, R.L.: Vitamin D-intoxicated patient with hypoparathyroidism: hypercalcemia, acute cerebral ataxia, and EEG changes: magnesium sulfate therapy. Arch. intern. Med. **114**, 375 (1964).

MAURICE, P.F., HENNEMAN, P.H.: Medical aspects of renal stones. Medicine (Baltimore) **40**, 315 (1960).

MAYOR, G.: Nierensteine und Hyperparathyreoidismus. Urol. int. (Basel) **13**, 294 (1962).

MCCARTY, D.J.: Pseudogout; articular chondrocalcinosis. Calcium pyrophosphate crystal deposition disease. In: HOLLANDER, J.L. (ed.), Arthritis and allied conditions, p. 947. Philadelphia: Lea & Febiger 1966.

— HOGAN, J.M., GATTER, R.A., GROSSMAN, N.: J. Bone Jt Surg. A **48**, 309 (1966).

— KOHN, N.N., FAIRES, J.S.: The significance of calcium phosphate crystals in the synovial fluid of arthritic patients. The "pseudogout syndrome". Ann. intern. Med. **56**, 711, 738 (1962).

MCGEOWN, M.G.: Effect of parathyroidectomy on the incidence of renal calculi. Lancet **1961 I**, 586.

MELICK, R.A., HENNEMAN, P.H.: Clinical and laboratory studies of 207 consecutive patients in a kidney-stone clinic. New Engl. J. Med. **259**, 307 (1958).

MELVIN, K.: Articular chondrocalcinosis, hyperparathyroidism and pseudogout: hypomagnesiemic crisis. Proc. roy. Soc. Med. **59**, 595 (1966).

MELVIN, K.E.W., CASTLEMAN, B.: Case records of the Massachusetts General Hospital (Case 50-1971), Persistent hypercalcemia after removal of a parythyroid tumor. New Engl. J. Med. **285**, 1422 (1971).

MINDER: Besondere Befunde bei der Parathyreotoxikose. Schweiz. med. Wschr. **87**, 667 (1957).

MINTZ, D.H., CANARY, J.J., CAMERON, G., KYLE, L.H.: Hyperuricaemia in hyperparathyroidism. New Engl. J. Med. **265**, 115 (1961).

MIXTER, C.G., KEYNES, W.M., COPE, O.: Further experience with pancreatitis as a diagnostic clue to hyperparathyroidism. New Engl. J. Med. **266**, 265 (1962).

MURPHY, T.R., RE MINE, W.H., BURBASCK, M.K.: Hyperparathyroidism: report of a case with parathyroid adenoma presented primarily with profound muscular weakness. Proc. Mayo Clin. **35**, 629 (1960).

PALOYAN, E.: Recent developments in the early diagnosis of hyperparathyroidism. Surg. Clin. N. Amer. **47**, 61 (1967).

— LAWRENCE, A.M., STRAUS, F.H., PALOYAN, D., HARPER, P.V., CUMMINGS, D.: Alpha-cell hyperplasia in calcific pancreatitis associated with hyperparathyroidism. J. Amer. med. Ass. **200**, 757 (1957).

— PALOYAN, D., HARPER, P.V.: The role of glucagon hypersecretion in the relationship of pancreatitis and hyperparathyroidism. Surgery **62**, 167 (1967).

PETERSEN, P.: Die Psychiatrie des primären Hyperparathyreoidismus. Berlin-Heidelberg-New York: Springer 1967.

— Psychiatric disorders in primary hyperparathyroidism. J. clin. Endocr. **28**, 1491 (1969).

PRADER, A., UEHLINGER, E., ILLIG, R.: Hypercalcämie bei Morbus Addison im Kindesalter. Helv. paediat. Acta **14**, 607 (1959).

PYRAH, L.N., HODGKINSON, A., ANDERSON, C.K.: Primary hyperparathyroidism. Brit. J. Surg. **53**, 245 (1966).

RAMSDELL, J.A., BLACK, B.M., WOOLNER, L.B., KEATING, F.R., JR.: Tumors of parathyroid glands found incidentally during surgical procedures. Surg. Gynec. Obstet. **111**, 451 (1960).

RANDALL, R.V., KEATING, F.R., JR.: Serendipity in the diagnosis of primary hyperparathyroidism. Amer. J. med. Sci. **236**, 575 (1958).

RASMUSSEN, H., REIFENSTEIN, E.C., JR.: In: Testbook of endocrinology, R.H. WILLIAMS, ed. Philadelphia: W.B. Saunders 1962.

REISS, E., CANTERBURY, J.M.: Primary hyperparathyroidism: Differentiation of adenoma and hyperplasia and localization of hyperfunctioning glands. New Engl. J. Med. **280**, 1381 (1969).

REITZ, R.E., POLLARD, J.J., WANG, C.A., FLEISCHLI, D.J., COPE, O., MURRAY, T.M., DEFTOS, L.J., POTTS, J.T.: Localization of parathyroid adenomas by selective venous catheterization and radioimmunoassay. New Engl. J. Med. **281**, 348, 1248 (1969).

RIGGS, B.L., JONES, I.D., RANDALL, R.V.: Primary hyperparathyroidism: report of a case in which the diagnosis was made by serendipity. Proc. Mayo Clin. **37**, 250 (1962).

RUTISHAUSER, E.: Hypophyseo-parathyreotisches Syndrom, Hypophysentumor, adenomatöse Epithelkörperchen-Hyperplasie, Ostitis fibrosa v. Recklinghausen. Zbl. allg. Path. path. Anat. **80**, 193 (1943).

SAMUELS, B.I., DOWDY, A.H., LECKY, J.W.: Localization of parathyroids by thermography. New Engl. J. Med. **286**, 216 (1972).

SCOTT, J.T., DIXON, A.ST.J., BYWATERS, E.G.L.: Association of hyperuricaemia and gout with hyperparathyroidism. Brit. med. J. **1964 I**, 1070.

SHAI, F., BAKER, R.K., ADDRIZZO, J.R., WALLACH, S.: Hypercalcemia in mycobacterial infection. J. clin. Endocr. **34**, 251 (1972).

SHERWOOD, L.M., O'RIODAN, J.L.H., AURBACH, G.D., POTTS, J.T.: Production of parathyroid hormone by nonparathyroid tumors. J. clin. Endocr. **27**, 140 (1967).

SLUYS VEER, J. VAN DER, BIRKENHÄGER, J.C., SMEENK, D.: De invloed van oraal toegediende diuretica op de calcium en fosfaat stofwisseling. Ned. T. Geneesk. **109**, 1795 (1965).

— — — The effect of orally administered diuretics on calcium metabolism. Fourth European Symposion on calcified tissues, p. 96. Excerpta Medica Foindation, 1966.

— SMEENK, D., HEUL, R.O. VAN DER: Tetracycline labelling of bone in hyperparathyroidism. In: BLACKWOOD, H.J.J., Bone and tooth, Proc. I. Europ. Sympos., p. 85. Oxford: Pergamon Press 1964.

SMEENK, D., SLUYS VEER, J. VAN DER, HEUL, R.O. VAN DER: Onderzoek van bot in vivo gemerkt met tetracycline inleiding. Ned. T. Geneesk. **107**, 2007 (1963).

STAMPL, B.: Die beidseitige Nierenvenenthrombose bei akutem Hyperparathyreoidismus. Frankfurt. Z. Path. **72**, 320 (1963).

STEINBACH, H.L., GORDAN, G.S., EISENBERG, E., CRANE, J.T., SILVERMAN, S., GOLDMAN, L.: Primary hyperparathyroidism: a correlation of roentgen, clinical, and pathologic features. Amer. J. Roentgenol. **86**, 329 (1961).

ST. GOAR, W.T.: Gastrointestinal symptoms as a clue to the diagnosis of primary hyperparathyroidism: a review of 45 cases. Ann. intern. Med. **46**, 102 (1957).

TURRIAN, H., MAIER, C.: Über die Kombination von Arthritis urica und Adenom der Glandula parathyreoidea. Schweiz. med. Wschr. **96**, 1306 (1966).

WELTI, H.: A propos de deux observations de localisation maxillaire de l'ostéose parathyroïdienne. Bull. schweiz. Akad. med. Wiss. **18**, 285 (1963).

WERNLY, M.: Hyperparathyreoidismus und Niereninsuffizienz. Beitrag zur Kenntnis der Nephrocalcinose. Z. klin. Med. **140**, 226 (1942).

— BERDJIS-CHAMSI, CH.: Les parathyroides humaines. Basel: Schwabe 1946.

— Die klinischen Formen des primären Hyperparathyreoidismus. Urol. int. (Basel) **19**, 93 (1965).

— Diagnostik der Parathyreoideaerkrankungen. Schweiz. med. Wschr. **97**, 1234 (1967).

— KÖNIG, M.P.: Hypercalcämie-Syndrom und Hypercalciurie. Schweiz. med. Wschr. **91**, 795 (1961).

WILLS, M. R., PAK, C. Y. C., HAMMOND, W. G., BARTTER, F. C.: Normocalcemic primary hyperparathyroidism. Amer. J. Med. **47**, 384 (1969).
WILSON, R. E.: Diskussionsbemerkung zu PALOYAN et al. Surgery **62**, 172 (1967).
WILTSE, H. E., GOLDBLOOM, R. B., ANTIA, A. U., OTTESEN, O. E., ROWE, R. D., COOKE, R. E.: Infantile hypercalcemia syndrome in twins. New Engl. J. Med. **275**, 1157 (1966).
YENDT, E. R., GAGNÉ, R. J. A., COHANIM, M.: The effects of thiazides in idiopathic hypercalciuria. Amer. J. Med. Sci. **251**, 449 (1966).

Prognosis and Treatment (Including Postoperative Treatment)

ALBRIGHT, F., REIFENSTEIN, E. C.: The parathyroid glands and metabolic bone disease. Baltimore: Williams & Wilkins Co. 1948.
BLACK, B. M.: The pathology and surgery of the parathyroid glands. In: GREEP, R. O., and R. V. TALMAGE, The parathyroids. Springfield: Ch. C. Thomas 1961.
— Hyperparathyroidism. Springfield: Ch. C. Thomas 1953.
BREUER, R. I., LEBAUER, J.: Caution in the use of phosphates in treatment of severe hypercalcemia. J. clin. Endocr. **27**, 695 (1967).
CHAKMAKJIAN, Z. H., BETHUNE, J. E.: Sodium sulfate treatment of hypercalcemia. New Engl. J. Med. **275**, 862 (1966).
CHURCHILL, E. D., COPE, O.: Parathyroid tumors associated with hyperparathyroidism: 11 cases treated by operation. Surg. Gynec. Obstet. **58**, 255 (1934).
COPE, O.: Surgery of hyperparathyroidism: the occurrence of parathyroids in the anterior mediastinum and the division of the operation into two stages. Ann. Surg. **114**, 706 (1941).
— The story of hyperparathyroidism at the Massachusetts General Hospital. New Engl. J. Med. **274**, 1174 (1966).
GOLDSMITH, R. E., ZALME, E., WEINSTEIN, A. S.: Clinical experience with primary hyperparathyroidism: evaluation of treatment. Acta endocr. (Kbh.) **52**, 221 (1966).
GOLDSMITH, R. S., INGBAR, S. H.: Inorganic phosphate treatment of hypercalcemia of diverse etiologies. New Engl. J. Med. **274**, 1 (1966).
FEIND, C. R.: Re-exploration for parathyroid adenoma. Amer. J. Surg. **108**, 543 (1964).
KISTLER, H. J.: Treatment of hypercalcemia with inorganic phosphate. Helv. med. Acta **33**, 447 (1967).
— NEUBAUER, W.: Phosphatbehandlung bei Hypercalcämie. Klin. Wschr. **48**, 741 (1970).
LEROY, H.: Hypoparathyroidism requiring massive amounts of medication, with apparent response to magnesium sulfate. J. clin. Endocr. **21**, 219 (1961).
LYNCH, H. T., LEMON, H. M., HENN, M. J., ELLINGSON, R. J., GRISSOM, R. L.: Vitamin D-intoxicated patient with hypoparathyroidism: hypercalcemia, acute cerebral ataxia, and EEG changes: magnesium sulfate therapy. Arch. intern. Med. **114**, 375 (1964).
MAYOR, G.: Indication opératoire, traitement chirurgical et traitement postopératoire. Urol. int. (Basel) **19**, 131 (1965).
MELVIN, K.: Articular chondrocalcinosis, hyperparathyroidism and pseudogout: hypomagnesiemic crisis. Proc. roy. Soc. Med. **59**, 595 (1966).
SHACKNEY, S., HASSON, J.: Precipitous fall in serum calcium, hypotension and acute renal failure after intravenous phosphate therapy for hypercalcemia. Report of 2 cases. Ann. intern. Med. **66**, 906 (1967).
SINGER, F. R., NEER, R. M., MURRAY, T. M., KEUTMANN, H. T., DEFTOS, L. J., POTTS, J. T.: Mithramycin treatment of intractable hypercalcemia due to parathyroid carcinoma. New Engl. J. Med. **283**, 634 (1970).
SUKI, W. N., YIUM, J. J., VON MINDEN, M., SALLER-HEBERT, C., EKNOYAN, G., MARTINEZ-MALDONADO, M.: Acute treatment of hypercalcemia with furosemide. New Engl. J. Med. **283**, 836 (1970).
WILSON, D. R., YENDT, E. R.: Treatment of the adult Fanconi syndrome with oral phosphate supplements and alkali: report of two cases with nephrolithiasis. Amer. J. Med. **35**, 487 (1963).

Secondary Hyperparathyroidism

ANDERSON, W. W., MANN, J. B., KENYON, N., FARELL, J. J., HILLS, G.: Subtotal parathyroidectomy in acotemic renal osteodystrophy. New Engl. J. Med. **268**, 575 (1963).
ARNAUD, C., RASMUSSEN, H., ANAST, C.: Further studies on the interrelationship between parathyroid hormone and vitamin D. J. clin. Invest. **45**, 1955 (1966).
BALL, J.: Diseases of bone. In: Recent advances in pathology, p. 293 (C. V. HARRISON, ed.). London: J. & A. Churchill 1960.
BERNER, A.: Les ostéodystrophies d'origine rénale. Helv. med. Acta **10**, 741 (1944).
BERSON, S. A., YALOW, R. S.: Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia and bronchogenic carcinoma. Science **154**, 907 (1966).
BINSWANGER, U., FISCHER, J. A.: Stimulation of parathyroid hormone secretion by intravenous phosphorus infusion in primary hyperparathyroidism. Klin. Wschr., in press (1973).
BUCKLE, D. M.: Hyperparathyroidism in chronic renal failure. Assessment of autonomy by plasma-parathyroid-hormone response to alterations in Calcium. Lancet **1970 II**, 234.
CARE, A. D., SHERWOOD, L. M., POTTS, J. T., AURBACH, G. D.: Perfusion of the isolated parathyroid gland of the goat and sheep. Nature (Lond.) **209**, 55 (1966).
CASTLEMAN, B., MALLORY, T. B.: Parathyroid hyperplasia in chronic renal insufficiency. Amer. J. Path. **13**, 553 (1937).
COPP, D. H., CAMERON, E. C., CHENEY, B. A., DAVIDSON, A. G. F., HENZE, K. G.: Evidence for calcitonin. — A new hormone from the parathyroid that lowers blood calcium. Endocrinology **70**, 638 (1962).
CRABBÉ, P., ISSELBACHER, K. J.: Urinary hydroxyproline excretion in malabsorption states. Gastroenterology **48**, 307 (1965).
FISCHER, J. A., BINSWANGER, U., SCHENK, R., MERZ, W., KISTLER, H. J.: Normocalcemic secondary hyperparathyroidism and malabsorption. Helv. med. Acta **35**, 30 (1969).
FOLLIS, R. H., JACKSON, D. A.: Renal osteomalacia and osteitis fibrosa in adults. Bull. Johns Hopk. Hosp. **72**, 232 (1943).
GOLDMAN, R., BASSET, S. H.: Phosphorus excretion in renal failure. J. clin. Invest. **33**, 1623 (1954).
GOLDSMITH, R. S., FURSZYFER, J., JOHNSON, W. J., FOURNIER, A. E., ARNAUD, C. D.: Control of secondary hyperparathyroidism during long term hemodialysis. Amer. J. Med. **50**, 692 (1971).
HANNA, S., HARRISON, M., MACINTYRE, I., FRASER, R.: The syndrome of magnesium deficiency in man. Lancet **1960 II**, 172.
— NORTH, K. A. K., MACINTYRE, I., FRASER, R.: Magnesium metabolism in parathyroid disease. Brit. med. J. **1961 II**, 1953.
HEATON, F. W.: The parathyroid glands and magnesium metabolism in the rat. Clin. Sci. **28**, 543 (1965).
KATZ, A. I., HAMPERS, C. L., MERRILL, J. P.: Secondary hyperparathyroidism and renal osteodystrophy in chronic renal failure. Medicine (Baltimore) **48**, 333 (1969).
MACH, R. S., RUTISHAUSER, E.: Les ostéodystrophies rénales. Etude expérimentale et anatomoclinique des lésions osseuses au cours des néphrites. Helv. med. Acta **4**, 423 (1937).

MELVIN, K.E.W., CASTLEMAN, B.: Case records of the Massachusetts General Hospital (Case 50-1971), Persistent hypercalcemia after removal of a parathyroid tumor. New Engl. J. Med. **285**, 1422 (1971).
MACINTYRE, I., BOSS, S., TROUGHTON, V.A.: Parathyroid hormone and magnesium homeostasis. Nature (Lond.) **198**, 1058 (1963).
MCINTOSH, D.A., PETERSON, E.W., MCPHAUL, J.J.: Autonomy of parathyroid function after renal homotransplantation. Ann. intern. Med. **65**, 900 (1966).
MCPHAUL, J.J., MCINTOSH, D.A., HAMMOND, W.S., PARK, O.K.: Autonomous secondary (renal) parathyroid hyperplasia. New Engl. J. Med. **271**, 1342 (1964).
NORDIN, B.E.C., FRASER, R.: The indirect assessment of parathyroid function. In: WOLSTENHOLME, G.E.W. (ed.), Ciba Foundation Symposium on bone structure and metabolism, p. 222. London: J. & A. Churchill 1956.
— SMITH, D.A.: Diagnostic procedures in disorders of calcium metabolism, p. 37. London: J. & A. Churchill 1965.
O'RIORDAN, J.L.H., PAGE, J., KERR, D.N.S., WALES, J., MOORHEAD, J., CROCKETT, R.E., FRANZ, H., RITZ, E.: Hyperparathyroidism in chronic renal failure and dialysis osteodystrophy. Quart. J. Med. **39**, 359 (1970).
PARFITT, A.M.: Relation between parathyroid cell rass and plasma calcium concentration in normal and uremic subjects. Arch. intern. Med. **124**, 269 (1969).
SCHWARTZ, E., PANARIELLO, V.A., SAEL, J.: Radioactive calcium kinetics during high calcium intake in osteoporosis. J. clin. Invest. **44**, 1547 (1965).
SHERWOOD, L.M., CARE, A.D., MAYER, G.P., AURBACH, G.D.: Evaluation by radioimmunoassay of factors controlling the secretion of parathyroid hormone. Nature (Lond.) **209**, 52 (1966).
STANBURY, S.W.: Bony complications of renal disease. In: Renal disease, ed. by D.A.K. BLACK. Oxford: Blackwell Scientific Publications 1962.
— The treatment of renal osteodystrophia. Ann. intern. Med. **65**, 1133 (1966).
— LUMB, G.A.: Metabolic studies of renal osteodystrophy. Medicine (Baltimore) **41**, 1 (1962).
— — NICHOLSON, W.F.: Elective subtotal parathyroidectomy for renal hyperparathyroidism. Lancet **1960I**, 793.
TALMAGE, R.V., TOFT, R.J.: The problem of the control of parathyroid secretion. In: GREEP, R.O., and R.C. TALMAGE, The parathyroids, p. 224. Springfield: Ch. C. Thomas 1961.
TORRIANI, PH.: Le role de l'endoste dans les cachexies et dans l'ostéomalacie sénile. Diss. Med. Genève, 1924.
VOSIK, W.M., ANDERSON, C.F., STEFFEES, W.P., JOHNSON, W.J., ARNAUD, C.D., GOLDSMITH, R.S.: Successful medical management of osteitis fibrosa due to "tertiary" hyperparathyroidism. Mayo Clinic Proc. **47**, 110 (1972).
WERNLY, M., BERDJIS-CHAMSI, CH.: Les parathyroïdes humaines. Basel: Benno Schwabe & Co. 1946.
WILSON, R.E., BERNSTEIN, D.S., MURRAY, J.E., MOORE, F.D.: Effects of parathyroidectomy and kidney transplantation on renal osteodystrophy. Amer. J. Surg. **110**, 384 (1965).

Normocalcemic Secondary Hyperparathyroidism

ARNAUD, C.D., GLORIEUX, F., SCRIVER, C.R.: Serum parathyroid hormone levels in acquired vitamin D deficience of infancy. Pediatrics **49**, 837 (1972).
FISCHER, J.A., BINSWANGER, U., FANCONI, A., ILLIG, R., BAERLOCHER, K., PRADER, A.: Serum parathyroid hormone concentrations in vitamin D deficiency. Horm. Metab. Res. **5**, in press (1973).
— — SCHENK, R., MERZ, W., KISTLER, H.-J.: Normocalcemic secondary hyperparathyroidism and malabsorption. Helvet. med. Acta **35**, 30 (1969).

Tertiary Hyperparathyroidism

BRICKER, N.S., FORREST, J., AVIOLI, L., SLATOPOLSKY, E., KISSANE, J., CHASE, L., LUBOWITZ, H.: Tertiary hyperparathyroidism—does it exist? Amer. J. Med. **52**, 254 (1972).
DAVIES, D.R., DENT, C.E., WILLCOX, A.: Hyperparathyroidism and steatorrhoea. Brit. med. J. **1956II**, 1133.
GOLDEN, A., CANARY, J.J., KERWIN, D.M.: Concurrence of hyperplasia and neoplasia of the parathyroid glands. Amer. J. Med. **38**, 562 (1965).
GOLDSMITH, R.S., FURSZYFER, J., JOHNSON, W.J., FOURNIER, A.E., ARNAUD, C.D.: Control of secondary hyperparathyroidism during long term hemodialysis. Amer. J. Med. **50**, 692 (1971).
HERDMAN, R.C., MICHAEL, A.F., VERNIER, R.L., KELLY, W.D., GOOD, R.A.: Renal function and phosphate excretion after human renal homotransplantation. Lancet **1956I**, 121.
LUDWIG, G.D., KYLE, G.C., DEBLANCO, M.: "Tertiary" hyperparathyroidism induced by osteomalacia resulting from phosphorus depletion. Amer. J. Med. **43**, 136 (1967).
MCINTOSH, D.A., PETERSON, E.W., MCPHAUL, J.J.: Anatomy of parathyroid function after renal homotransplantation. Ann. intern. Med. **65**, 900 (1966).
ST. GOAR, W.T.: Case record 29-1963, Massachusetts General Hospital. New Engl. J. Med. **268**, 949 (1963).
VOSIK, W.M., ANDERSON, C.F., STEFFEES, W.P., JOHNSON, W.J., ARNAUD, C.D., GOLDSMITH, R.S.: Successful medical management of osteitis fibrosa due to "tertiary" hyperparathyroidism. Mayo Clin. Proc. **47**, 110 (1972).
WILSON, R.E., BERNSTEIN, D.S., MURRAY, J.E., MOORE, F.D.: Effects of parathyroidectomy and kidney transplantation on renal osteodystrophy. Amer. J. Surg. **110**, 384 (1965).

Laboratory and Function Tests for Diseases of the Parathyroids

Reviews

COURVOISIER, B., JEANRENAUD, B., JENNY, M.: Diagnostic clinique des affections parathyroïdiennes. Docum. Geigy Acta clin. **2**, 83 (1963).
FRASER, R.: Primary hyperparathyroidism. Lancet **1961II**, 1256.
GORDAN, G.S., EISENBERG, E., LOKEN, H., GARDNER, B., HAYASHIDA, T.: Clinical endocrinology of parathyroid hormone excess. Recent Progr. Hormone Res. **18**, 297 (1962).
HAAS, H.G.: Knochenstoffwechsel und Parathyreoidea-Erkrankungen. Stuttgart: Georg Thieme 1966.
— Metabolic criteria for primary hyperparathyroidism. Helv. med. Acta **33**, 91 (1966).
HODGKINSON, A.: Biochemical aspects of primary hyperparathyroidism. Clin. Sci. **25**, 231 (1963).
HOWARD, J.E.: Clinical disorders of calcium homeostasis. Medicine (Baltimore) **42**, 25 (1963).
KEATING, F.J.: Diagnosis of primary hyperparathyroidism. Clinical and laboratory aspects. J. Amer. med. Ass. **178**, 547 (1961).
KLOTZ, H.P., CLEMENT, D.: Les tests dynamiques d'exploration parathyroïdienne. Path. Biol. **9**, 291 (1961).
LICHWITZ, A., PARLIER, R.: Calcium et maladies métaboliques de l'os (3 vol.). Paris: L'expansion éditeur 1964.
NORDIN, B.E.C., SMITH, D.A.: Diagnostic procedures in disorders of calcium metabolism. London: J. & A. Churchill 1965.
RIDDICK, F.A., REISS, E.: Hyperparathyroidism. Analysis of recent experiences. Clinical spectrum and diagnostic tests. Ann. intern. Med. **56**, 183 (1962).

Laboratory Methods and Tests

ARNAUD, C. D., TSAO, H. S., LITTLEDIKE, T.: Radioimmunoassay of human parathyroid hormone in man. J. clin. Invest. **50**, 21 (1971).

BERSON, S. A., YALLOW, R. S.: Parathyroid hormone in plasma in adenomatous hyperparathyroidism, uremia and bronchogenic carcinoma. Science **54**, 907 (1966).

BIRKENHÄGER, J. C., SLUYS VEER, J. VAN DER, HEU, R. O., VAN DER, SMEENK, D.: Studies of iliac crest bone from controls and patients with bone disease by means of chemical analysis, tetracycline labelling and histology. In: FLEISCH, H., BLACKWOOD, H. J. J., OWEN, M. (ed.), Third European Symp. on Calcified Tissues, p. 73. Berlin-Heidelberg-New York: Springer 1966.

BLAU, M., NAGLER, W., BENDER, M. A.: Fluorine-18, a new isotope for bone scanning. J. nucl. Med. **3**, 332 (1962).

COMAR, C. L., WASSERMAN, R. H.: Strontium. In: COMAR, C. L., BRONNER, F. (eds.), Mineral metabolism, an advanced treatise, vol. II, p. 523. New York, London: Academic Press 1964.

COREY, K. R., KENNY, P., GREENBERG, E., LAUGHLIN, J. S.: Detection of bone metastases in scanning studies with calcium-47 and strontium-85. J. nucl. Med. **3**, 454 (1962).

DILLA, M. A. VAN, ARNOLD, J. S.: Strontium 85 tracer studies in humans. Int. J. appl. Radiat. **1**, 129 (1956).

ELIEL, L. P., CHANES, R., HAWRYLKO, J.: Urinary excretion of parathyroid hormone in man. J. clin. Endocr. **25**, 445 (1965).

FANCONI, A., ROSE, G. A.: The ionized, complexed and protein-bound fractions of calcium in plasma. Quart. J. Med. **27**, 463 (1958).

GRUNDIG, E., CZITOBER, H., SCHOBEL, B.: Vergleichende Untersuchungen von „sauren" Plasmaphosphatasen bei verschiedenen Knochenerkrankungen. Clin. chim. Acta **12**, 157 (1965).

HAAS, H. G.: Die Abklärung von Knochenkrankheiten. Internist (Berl.) **7**, 558 (1966).

HEANEY, R. P.: Evaluation and interpretation of calcium-kinetic data in man. Clin. Orthop. **30** (1963).

ISAKSSON, B., SJÖGREN, B.: Errors inherent in metabolic balance studies. In: Proc. second internat. congr. endocrinol., p. 1041. Amsterdam, New York: Excerpta Med. Found. 1964.

KOFMAN, S., SKY-PECK, H. H., THIBAUDEAU, Y., RAY, R. D., TAYLOR, S. G.: The use of strontium-85 in the evaluation of bone metastases: a preliminary report. J. nucl. Med. **4**, 9 (1963).

LAFFERTY, F. W., PEARSON, O. H.: Skeletal intestinal and renal calcium dynamics in hyperparathyroidism. J. clin. Endocr. **23**, 891 (1963).

LEOYD, H. M., ROSE, G. A.: Ionized, protein-bound and complexed calcium in the plasma in primary hyperparathyroidism. Lancet **1958 II**, 1258.

MCLEAN, F. C., BUDY, A. M.: Radiation, isotopes and bones. New York, London: Academic Press 1964.

Medical uses of Ca 47, IAEA technical reports series, No 10. Int. Atomic Energy Agency, Vienna (1962).

MILCH, R. A., RALL, D. P., TOBIE, J. E.: Fluorescence of tetracycline antibiotics in bone. J. Bone Jt Surg. A **40**, 897 (1958).

MILHAUD, G., AUBERT, J. P.: Exploration du métabolisme calcique chez l'homme à l'aide du calcium 45. Path. Biol. No V-VI, p. 405 (1958).

NORDIN, B. E. C., BLUHM, M., MACGREGOR, J.: In vitro and in vivo studies with bone-seeking isotopes. In: Radioisotopes and bone, p. 105. Oxford: Blackwell Scientific Publ. 1962.

POTTS, J. T., JR., DEFTOS, L. J.: Parathyroid hormone, thyrocalcitonin, vitamin D, bone and bone mineral metabolism. In: Duncan's diseases of metabolism, 6th ed. Philadelphia: W. B. Saunders 1969.

REISS, E., CANTERBURY, J. M.: A radioimmunoassay for parathyroid hormone in man. Proc. Soc. exp. Biol. (N. Y.) **128**, 501 (1968).

RIGGS, B. L., ARNAUD, C. D., REYNOLDS, J. C., SMITH, L. H.: Immunologic differentiation of primary hyperparathyroidism due to nonparathyroid camncer. J. clin. Invest. **50**, 2079 (1971).

ROSE, G. A.: Determination of the ionized and ultrafiltrable calcium of normal human plasma. Clin. chim. Acta **2**, 227 (1957).

SOLOMON, A. K.: Compartmental methods of kinetic analysis. In: Mineral metabolism, vol. I, p. 119. New York: Academic Press Inc. 1960.

Diagnosis of Primary Hyperparathyroidism

Calcium

DENT, C. E.: Some problems of hyperparathyroidism. Brit. med. J. **1962 II**, 1419.

EPSTEIN, F. H.: Calcium and the kidney. Amer. J. Med. **45**, 700 (1968).

MACINTYRE, I.: The flame-spectrophotometric determination of calcium in biologic fluids and an isotopic analysis of the errors in the Kramer-Tisdall procedure. Biochem. J. **67**, 164 (1957).

RITTER, S. H., SPENCER, H., SAMACHSON, J.: The Sulkowitch test and quantitative urinary calcium excretion. J. Lab. clin. Med. **56**, 1314 (1960).

WERNLY, M., KÖNIG, M. P.: Hypercalcämie-Syndrom und Hypercalciurie. Schweiz. med. Wschr. **91**, 795 (1961).

Urinary Hydroxyproline Excretion

HORWITH, M., BUCHANAN, J. R., GUDMUNDSSON, S. T., MAUTALEN, C. A.: Primary hyperparathyroidism. Evaluation of diagnostic aids: calcium infusion, phosphorus deprivation and hydroxyprolinuria. J. clin. Endocr. **26**, 885 (1966).

KOCHER, P.: Etude de l'hydroxyprolinurie dans les ostéopathies. Path. Biol. **14**, 1020 (1966).

PROCKOP, D. J., KIVIRIKKO, K. I.: Relationship of hydroxyproline excretion in urine to collagen metabolism. Ann. intern. Med. **66**, 1243 (1967).

SMILEY, J. D., ZIFF, M.: Urinary hydroxyproline excretion. Physiol. Rev. **44**, 30 (1964).

Phosphate Clearance, Tubular Reabsorption of Phosphate, Phosphate Excretion Index, Maximal Tubular Reabsorption of Phosphate

BERNSTEIN, D., KLEEMAN, C. R., ROCKNEY, R., DOWLING, J. T., MAXWELL, M. H.: Studies on the renal clearance of phosphate and the rate of the parathyroid glands in its regulation. J. clin. Endocr. **22**, 641 (1962).

BIJOVET, O. L. M.: Relation of plasma phosphate concentration to renal tubular reabsorption of phosphate. Clin. Sci. **37**, 23 (1969).

— MORGAN, D. B., FOURMAN, P.: The assessment of phosphate reabsorption. Clin. chim. Acta **26**, 15 (1969).

HODGKINSON, A.: Renal phosphate excretion indices in the diagnosis of hyperparathyroidism. Clin. Sci. **21**, 125 (1961).

HORWITH, M., BUCHANAN, J. R., GUDMUNDSSON, S. T., MAUTALEN, C. A.: Primary hyperparathyroidism. Evaluation of diagnostic aids: calcium infusion, phosphorus deprivation and hydroxyprolinuria. J. clin. Endocr. **26**, 885 (1966).

MORGAN, D. B.: Calcium and phosphate metabolism and the bone. Progr. Surg. **10**, 26 (1972).

NORDIN, B. E. C.: Diagnosis of primary hyperparathyroidism. Proc. roy. Soc. Med. **54**, 641 (1961).
— BULUSU, L.: A modified index of phosphate excretion. Postgrad. med. J. **44**, 93 (1968).
— FRASER, R.: Assessment of urinary phosphate excretion. Lancet **1960 I**, 947.
— SMITH, D. A.: Diagnostic procedures in disorders of calcium metabolism. London: J. & A. Churchill 1965.
ROBINSON, C. J., MARTIN, T. J., MACINTYRE, I.: Phosphaturic effect of thyrocalcitonin. Lancet **1966 II**, 83.
SCHAAF, M., KYLE, L. H.: Measurement of per cent renal phosphorus reabsorption in the diagnosis of hyperparathyroidism. Amer. J. med. Sci. **228**, 262 (1954).
SIROTA, J. H.: Renal tubule reabsorption of phosphate in hyperparathyroidism before and after removal of parathyroid adenoma. Fed. Proc. **12**, 133 (1953).
STANBURY, S. W.: Some aspects of disordered renal tubular functions. Advanc. intern. Med. **9**, 231 (1958).

Suppression of Parathyroid Hormone Secretion with Calcium Infusions

HAAS, H. G., CANARY, J. J., KYLE, L. H., MINTZ, D. H.: Calcium infusion in the detection of bone disease in parathyroid disorders. Acta endocr. (Kbh.) **43**, 170 (1963).
HOWARD, J. E., HOPKINS, T. R., CONNOR, T. B.: On certain physiologic responses to i-v injection of calcium salts into normal, hyperparathyroid and hypoparathyroid persons. J. clin. Endocr. **13**, 1 (1953).
KYLE, L. H., ERDMAN, L. A., SCHAAF, M.: Investigation of a calcium infusion technic in the study of metabolic bone disease. Amer. J. Med. **14**, 754 (1953).
NORDIN, B. E. C., FRASER, R.: The effect of intravenous calcium on phosphate excretion. Clin. Sci. **13**, 477 (1954).
PAK, CH. Y. C., EAST, D., SANZENBACHER, L., RUSKIN, B., COX, J.: A simple and reliable test for the diagnosis of hyperparathyroidism. Arch. intern. Med. **129**, 48 (1972).

Stimulation of the Tubular Reabsorption of Phosphate with Parathyroid Extract (According to BECKER)

BECKER, K. L., PURNELL, D. C., JONES, J. D.: Tubular reabsorption of phosphate in primary hyperparathyroidism before and after administration of parathyroid hormone. J. clin. Endocr. **24**, 347 (1964).
COURVOSIER, B., BERTHOUD, E., ZAHND, G., GRANDJEAN, A.: Données actuelles sur la physiologie et la pathologie clinique des parathyroïdes. Helv. med. Acta **26**, Suppl. 38 au No. 5 (1959).

Cortisol Test (after DENT) for the Differential Diagnosis of a Hypercalcemia

ANDERSON, J., DENT, C. E., HARPER, C., PHILPOT, G. R.: Effect of cortisone on calcium metabolism in sarcoidosis with hypercalcemia. Lancet **1954 II**, 720.
BURR, J. M., FARRELL, J. J., HILLS, A. G.: Sarcoidosis and hyperparathyroidism with hypercalcemia. Special usefulness of the cortisone test. New Engl. J. Med. **261**, 1271 (1959).
DENT, C. E.: Cortisone test for hyperparathyroidism. Brit. med. J. **1956 I**, 230.
GWINUP, G., SAYLE, B.: Cortisone responsive hypercalcemia in proved hyperparathyroidism. Ann. intern. Med. **55**, 1001 (1961).
SHULMAN, L. E., SCHOENRICH, E. H., HARVEY, A. M.: Effects of adrenocorticotropic hormone (ACTH) and cortisone on sarcoidosis. Bull. Johns Hopk. Hosp. **91**, 371 (1952).
THOMAS, W. C., JR., CONNOR, T. B., MORGAN, H. G.: Some observations on patients with hypercalcemia exemplifying problems in differential diagnosis, especially in hyperparathyroidism. J. Lab. clin. Med. **52**, 11 (1958).
— — — Diagnostic considerations in hypercalcemia. New Engl. J. Med. **260**, 591 (1959).

Scintiscan Detection of a Parathyroid Adenoma after Radiolabeling

PALOYAN, E.: Rectent developments in the early diagnosis of hyperparathyroidism. Surg. Clin. N. Amer. **47**, 61 (1967).
POTCHEN, E. J.: The preoperative identification of the abnormal parathyroid—current status. Radiology **88**, 1170 (1967).

Diagnosis of Hypoparathyroidism

Ellsworth-Howard Test

COURVOISIER, B., BERTHOUD, E., ZAHND, G., GRANDJEAN, A.: Données actuelles sur la physiologie et la pathologie clinique des parathyroïdes. Helv. med. Acta **26**, Suppl. 38 au No. 5 (1959).
ELLSWORTH, R., HOWARD, J. E.: Studies on the physiology of the parathyroid glands; some responses of normal human kidneys and blood to intravenous parathyroid extract. Bull. Johns Hopk. Hosp. **55**, 296 (1934).
LUBELL, D.: The spontaneous diurnal variation of urinary phosphate excretion and its relation to the parathyroid hormone test. Helv. paediat. Acta **12**, 179 (1957).

Differential Diagnosis of the Hypocalcemia (Compare with Section on Pancreatitis in the chapter on Primary Hyperparathyroidism)

HALL, T. C., GRIFFITHS, C. T., PETRANEK, J. R.: Hypocalcemia—an unusual metabolic complication of breast cancer. New Engl. J. Med. **275**, 1474 (1966).

Stimulation of Parathyroid Hormone Secretion (Sodium Phytate, EDTA)

FOURMAN, P.: Calcium metabolism and the bone. Oxford: Blackwell Scientific Publications 1960.
JONES, K. H., FOURMAN, P.: Edetic acid test of parathyroid insufficiency. Lancet **1963 II**, 119.
ROSE, N.: Investigation of post thyroidectomy patients for hypoparathyroidism. Lancet **1963 II**, 116.
STOWERS, J. M., MICHIE, W., FRAZER, S. C.: A critical evaluation of the trisodium-edetate test for hypoparathyroidism after thyroidectomy. Lancet **1967 I**, 124.

Bone biopsy

BURKHARDT, R.: Knochenbiopsie. Internist (Berl.) **11**, 351 (1970).

XV. Tissue Hormones

A. LABHART

With Contributions by

CHR. HEDINGER and J. MÜLLER

A. Definition

Endogenous organic transmitter substances not formed in glands but in individual cells, which may be contained within organ tissues or scattered throughout the entire body, are described as tissue hormones (FELDBERG, 1955). They can reach their end organs either by the blood stream as in the case of gastrointestinal hormones, or by diffusion into their immediate surroundings (local hormones) (GADDUM, 1955). The term "cellular hormones", however, indicating substances which regulate metabolic processes within the cell, is still inadequately explained and it is better to use a different term. According to the definition, neurohormones are also tissue hormones. For practical reasons they are dealt with here in the chapters on the corresponding endocrine systems.

Finally, the hormones which are formed in tumors and produce the paraneoplastic syndromes are considered either as pathologic tissue hormones or as ectopically formed hormones (Chap. XVI).

The science of the tissue hormones today extends for the main part beyond clinical endocrinology and forms a special field of pharmacology and physiology. Those tissue hormones which are of no importance in clinical endocrinology are therefore only mentioned here in connection with more recent pertinent literature.

Three well-defined hyperfunctional syndromes of tissue hormones are known and are discussed below. Hypofunctional syndromes may also occur (COHEN, 1973), but need to be confirmed by further studies.

The tissue hormones can be divided according to their chemical nature into the following groups:

I. Polypeptide

1. Gastrointestinal hormones:
   a) Gastrin,
   b) Secretin,
   c) Cholecystokinin-pancreozymin,
   d) Cerulein,
   e) VIP (vasodilator intestinal peptide),
   f) GIP (gastric inhibitory peptide),
   g) Motilin.
   h) Intestinal glucagon
2. Kinins:
   a) Bradykinin,
   b) Kallidin,
   c) Methionine-kallidin.
3. Angiotensin II.
4. Substance P.
5. VDM, VEM.

II. Glycoproteins:

Erythropoietin.

III. Amines:

Serotonin, melatonin, dopamine.

IV. Fatty acids:

Prostaglandins.

The list of chemically identified gut hormones is almost certain to grow. The prime candidates are gastric inhibitory hormones from the intestine (enterogastrones) other than secretin and GIP. For other as yet hypothetical hormones see CROXATTO (1960).

B. Polypeptides

1. Gastrointestinal Hormones

Peristalsis and secretion in the digestive system are regulated partly by nervous influences, and partly by the mechanical, chemical and physicochemical effects of the chyme on the gastrointestinal mucosa. Signals from the receptors of the gastric and intestinal walls are transmitted to the glands involved in digestion mainly by way of the gastrointestinal hormones. These are formed in the gastrointestinal mucosa by cells which appear to have a common origin in the neural crest (APUD-cells, PEARSE, 1971) and

reach their end organs via the blood stream. They are all polypeptide in character.

Among the gastrointestinal hormones which are suspected and known, the structure of gastrin, secretin, cholecystokinin and pancreozymin (CCK-PZ), which seem to be identical, and GIP (gastric inhibitor polypeptide) have been clarified; the first two have been synthesized. The amino-acid composition and sequence of cerulein are known, and synthesis has been accomplished. The term entero-gastrone is now used for a group of gastrointestinal hormones with an inhibiting effect on gastric secretion.

A second glucagon ("enteroglucagon") which appears to be formed in the duodenal mucosa and not in the A cells of the islets is discussed in Chap. XIII (p. 822).

a) Gastrins

EDKINS suspected the existence of gastrin in 1905. KOMAROV demonstrated it in 1938, GREGORY and his team purified and synthesized it in 1964. Human gastrin I is a polypeptide of 17 amino acids. Gastrin II has the same actions, and is esterized with sulfuric acid at the 12th tyrosyl residue. Gastrin of pig, dog, and sheep differ from each other only in the 5th amino acid and perhaps the sixth. The end-terminal tetrapeptide, tyr–meth–asp–phe–NH_2, has the same qualitative action, although to a slighter degree, as the heptadeca polypeptide.

Gastrin is formed in the mucosa of the antrum and is released through mechanical stimuli (distension), chemical stimuli (proteins and their metabolites, alkaline pH), and indirectly through vagal stimulation and peristalsis. An acidic pH, below 1.5, and other, as yet unexplained, influences from the gut inhibit the secretion of gastrin.

Besides human gastrin I and II, at least three other types of gastrin have been described in man. The "big gastrins" (MW about 3900) contain the heptadeca peptide amide as a C-terminal sequence linked to the remainder of the molecule by a peptide bond (GREGORY, 1972; YAPON, 1971). They have a relatively slow turnover and – when compared on the basis of blood levels – less effect on gastric secretion than heptadeca gastrins (WALSH, 1973). An important source of big gastrin is the intestine. A rise of serum levels is observed after meals.

"Big big gastrins" have a molecular weight of about 20000. These gastrins constitute the major fraction of fasting gastrin in serum. They are elaborated by the gut and not released in response to a meal (YALOW, 1973). The biological actions of big gastrins are unknown.

Still another type of gastrin is found in patients with pernicious anemia. It may be determined by radioimmunoassay but is biologically inactive (HANSKY, 1973). Biological activity of gastrins is mainly determined by the end terminal tetrapeptide (tyr–meth–asp–phe–NH_2). The remainder of the molecule modifies the strength but not the type of this activity.

Recently the fasting concentration of gastrin in the serum has been determined radioimmunologically. Normal values differ in different laboratories, but the concentration is in the range of 20–150 pg/ml. Patients with ulcus duodeni have a significantly higher fasting value for gastrin than normals, patients with ulcus ventriculi, or patients in whom complete vagotomy has been performed (BYRNES, 1970). The same is true of patients with pernicious anemia (MCGUIGAN, 1970).

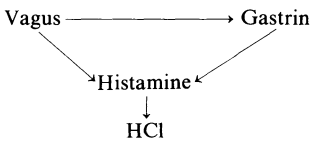


Fig. 1. Neural and humoral regulation of gastric acid secretion

When gastrin is given in physiological doses the following effects may be observed (GROSSMAN, 1970): stimulation of acid secretion and to a lesser extent pepsin secretion by the gastric mucosa, stimulation of water and enzyme secretion by the pancreas (this action probably does not occur in man), stimulation of bile flow, inhibition of water and electrolyte absorption in the intestine, contraction of the gastroesophageal sphincter, relaxation of the sphincter of Oddi, relaxation of the ileocecal sphincter, increased gastric mucosal blood flow, increased protein synthesis and growth in the gastrointestinal tract, contraction of gastric smooth muscle and release of insulin. There is still controversy as to which of these actions are of physiological importance and may also be observed following gastrin release by a meal. Pharmalogical actions following very high doses of exogenous gastrin include inhibition of gastric secretion, contraction of gallbladder and uterus and transient hypotension.

It has been proposed that the stimulatory effect of gastrin on acid secretion is mediated by histamine (CODE, 1965) (Fig. 1), which in turn stimulates adenyl cyclase. This may be true for amphibia but not for mammals (HAKANSON, 1971). Here, histamine might be one but is

certainly not the only mediator of gastrin action. Inactivation of gastric histamine receptors in man leads to a decrease but not to an abolition of gastrin stimulated acid secretion (BLACK, 1972).

Little is known about the mechanism of inactivation of gastrin. Liver and kidney seem to have a role in the degradation of gastrin. Serum gastrin rises proportionally in chronic renal failure with the serum creatinine. Whereas hemodialysis does not significantly alter gastrin levels, renal transplanation tends to return it to normal (KORMAN, 1972).

The synthetic pentapeptide, pentagastrin (butyloxycarbonyl–β-ala–tyr–meth–asp–phe–amide), can be used in a dose of 6 µg/kg body weight subcutaneously or 6 µg/h/kg by intravenous infusion or as snuff (1 mg every 10 minutes for 1 hour) (WORMSLEY, 1968) to produce the maximum stimulation for examination of the HCl production of the stomach. It has less side effects than histamine (multicenter pilot study 1967).

Other polypeptides found in animals with the same C-terminal pentapeptide as cerulein are even more active on the gastric secretion than gastrin (see p. 974). "Antigastrin" (2-phenyl-2-(2-pyridil)-thioacetamide) seems to be a competitive antagonist of gastrin (BEDI, 1967; OTTENJANN, 1968). In addition, antibodies against gastrin can be produced by crude gastrin extracts or gastrin coupled to a gamma-globulin (MC GUIGAN, 1968).

α) The Zollinger-Ellison Syndrome

The syndrome described in 1955 by ZOLLINGER and ELLISON includes the triad:

1. Severe, therapy-refractory ulcer disease, often with multiple (10%) ulcers, sometimes atypically located (25%), and with frequent complications.
2. Massive hypersecretion and hyperacidity of the gastric juice.
3. Non-B islet cell tumor of the pancreas, usually multiple, sometimes situated ectopically, and often (60%) malignant.

Watery diarrhea or steatorrhea often occurs in addition. The Zollinger-Ellison syndrome (ZES) results from overproduction of the tissue hormone gastrin. This syndrome must be differentiated from that of islet-cell tumors with diarrhea but no hyperacidity (see p. 974). Both syndromes have the same genetic basis. The ZES has recently been reviewed by ISENBERG (1973).

1. Frequency and Incidence. Up to 1964, 250 cases had been described. It is difficult to estimate the frequency, since individual cases are now seldom reported. At a rough estimate, 10% of unusually severe ulcer diseases are due to ZES (ZOLLINGER, 1964). The syndrome is more frequent in men (3:2) and occurs in every age group, but especially often in the 4th, 5th, and 6th decades; about 25% of cases of ZES are associated with adenomatosis (see Chap. XVIII). The combination of non-B islet cell adenomas and peptic ulcers in autopsy material chosen at random is more frequent than can be explained by the coincidence of probability (v. PLANTA, 1957).

Since multiple or atypical ulcers occur only in roughly one third of cases of ZES, tests for ZES should be performed in every case in which ulcer disease is refractory to therapy or has an atypical course, as also in cases with inexplicable diarrhea (HALLENBECK, 1968).

2. Morbid Anatomy and Histology. The tumors can be any size, from nodules visible only microscopically to tumors 8 cm in diameter, and are multiple in over 50% of cases. They are evenly distributed over the whole pancreas, but they can also arise ectopically (25%) in the duodenal mucosa or in the gastric antrum. Finally, development of the syndrome may also be due to adenomatosis or hyperplasia of the non-B islet cells (10%). Histologically, the cells are non-B islet cells. They probably arise from the argyrophil-metachromatic D cells (CAVALLIERO, 1967) although a few A-cell tumor have been described. There are signs of malignancy in over 50% of cases, and metastases are not uncommon, especially in the lymph nodes and liver (Fig. 2 from ZOLLINGER, 1964). The cells may have a trabecular, ribbon-like or an acinar-canalicular arrangement in which some authors see similaraties with pancreatic duct cells (CREUTZFELDT, 1973).

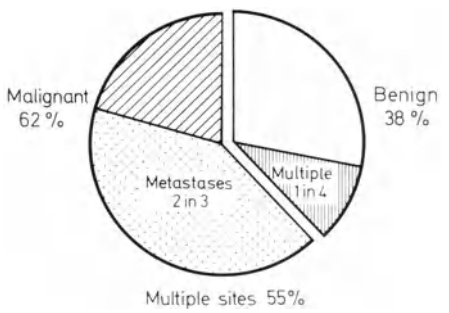


Fig. 2. Ulcerogenic tumors of the pancreas. Incidence of malignant, metastasizing multiple types (ZOLLINGER, 1964)

In the stomach there is hyperplasia of the parietal cells (MIEHER, 1962) which also extend to the antrum and are found in practically every

part of the stomach, probably due to chronic stimulation by gastrin. Hyperplastic glandular rods are demonstrable in the broadened hyperplastic folds of the gastric mucosa, which is reminiscent of Ménétrier's syndrome. Differentiation is, however, possible, as Ménétrier's syndrome is often associated with hyposecretion. Recently it seems that two types of ZES may be differentiated: type 1 with a short history, very high levels of serum gastrin, hyperplasia of G-cells in the gastric antrum and no pancreatic tumor, and type 2 less extreme hypergastrinemia and a tumor in the pancreatic islets (POLAK, 1972; Editorial, 1973). Still another type of ZES has been described where a gastric tumor arising from the antral G-cells producing gastrin caused the syndrome (ROYSTON, 1972).

3. Clinical Features. Ulcer symptoms dominate the picture in nine-tenths of cases and the remainder suffer from diarrhea. In the acute course the ulcers with complications are the presenting features. The disease can, however, also begin with diarrhea and loss of weight which may persist for some years before the ulcers become manifest (20%).

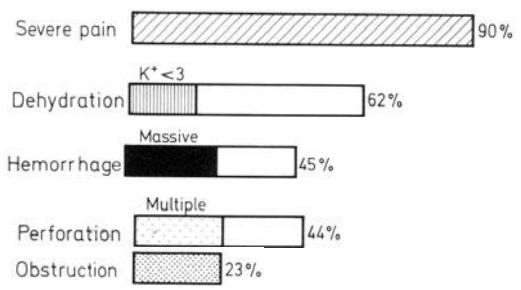


Fig. 3. Symptoms and complications of ZES

The ulcer most frequently (65% of cases) lies in the first two-thirds of the duodenum, and in a quarter of these just below the TREITZ's ligament. The pain is often constant and it does not respond to antacids. Ventricular ulcers are next in frequency, occurring in 20%. Jejunal peptic ulcers are characteristic of ZES, and ulcers are occasionally found in the esophagus. The ulcers are multiple in 12–15% of cases and all the complications, such as massive hemorrhage, perforation and obstruction, are common (Fig. 3, from ZOLLINGER, 1964). Ulcers regularly recur after conventional surgery. Although the symptoms of the ulcers in ZES are especially pronounced, they are not different in nature from those of the usual peptic ulcer.

Serum gastrin levels rise in proportion as the serum calcium increases. In the Zollinger-Ellison syndrome gastrin levels decrease after parathyroidectomy and rise again during calcium infusions, but do not respond to administration of parathormone (TRUDEAU, 1969).

Diarrhea in ZES (40%) is due to hypersecretion and hyperacidity and the direct stimulus is irritation of the small intestine by large amounts of acidic gastric juices. The diarrhea ceases if the gastric juices are continuously suctioned off. When the gastric juices are aspirated and neutralized and then given intraduodenally, they no longer cause diarrhea. The diarrhea is watery and reaches a volume of 5–10 liters/24 hours. It is refractory to opiates and leads to severe dehydration and in particular to hypokalemia, with grave or even fatal consequences.

The steatorrhea is also due to the hyperacidic hypersecretion and inactivation of the pancreatic lipase. As much as 30 g or more of fat may be excreted in the feces, and sometimes the full picture of a malabsorption syndrome can be present.

Islet-cell tumors rarely give rise to clinical symptoms. Even if malignancy is present the growth is slow and the course relatively benign.

4. Diagnosis. Before it was possible to estimate gastrin in the blood, the diagnosis of ZES was mainly based on the demonstration of HCl hypersecretion. The basal secretion, which is independent of vagal stimulation, is considerably raised in ZES, and is only slightly stimulated by histamine or appropriate stimulants. Estimation of the 12-hour secretion by continuous suction must produce a volume of more than 2 liters, with more than 100 mEq of free acid. One can never be certain that all the secretion has been obtained with a 12-hour estimation. A more reliable test is that of hourly basal secretion, which must yield a volume of more than 200 ml and an HCl content of over 20 mEq/h. The comparison of the basal secretion to the secretion after the maximum histamine test (Kay test) is of particular value. The production of acid after maximum histamine stimulation is proportional to the number of the parietal cells. The rise after maximum histamine or histalog stimulation should not be more than 60% and the ratio of the basal secretion to the maximum secretion in mEq HCl should be higher than 0.6. However, this test can also only give an indication of ZES since the basal secretion can vary considerably over the course of time, and the test may give false-positive or false-negative results (WINSHIP, 1967).

The most reliable diagnostic procedure now available is radioimmunological determination

of the fasting level of gastrin in the serum. The mean value differs from one laboratory to the other but is in the range of 20–150 pg/ml. In ZES the concentration is 10 times higher than normal, or even more (McGuigan, 1968). However, an increasing number of ZES with serum values below 300 pg/ml has been reported (Isenberg, 1973). Gastrin has been found in an islet-cell carcinoma with ZES in a concentration 16 times higher than in the serum of the patient, but there are gastrinomas which do not store gastrin to any significant degree.

Radiological examination of the gastrointestinal tract can detect the following characteristic findings in ZES:

1. Greatly increased fasting secretions, especially in the stomach, duodenum and proximal jejunum.
2. Frequently multiple ulceration, atypically situated (duodenum distal to the bulbus 19%, proximal jejunum 23%, several ulcers 10%).
3. Greatly hypertrophic stomach folds, edema in the duodenum and jejunum.
4. Megaduodenum.
5. Hypomotility of the stomach and duodenum with hypermotility of the small intestine (Amberg, 1964).

The tumors can sometimes be demonstrated angiographically by means of selective pancreas-arteriography (Clemett, 1967).

Detection of parietal cells in a biopsy specimen from the gastric mucosa in the antrum is further evidence of ZES.

The diagnosis is not difficult when there is a classic familial history, evidence of multiple and atypical ulcers, acid hypersecretion and hypergastrinemia. Surgery is definitely indicated in such cases. If, however, these pathognomonic features are not found in the history and radiological examination does not reveal adequate evidence for the disease, the following procedures may help in establishing a diagnosis:

1. Calcium infusion test (Passaro, 1970). In patients with ordinary duodenal ulcer, infusion of calcium (12–15 mg/kg over 3 hr period) produces an increase in acid secretion which is far below maximal acid output (MAO). In ZES, secretion may rise to MAO values.
2. Calcium injection test (Passaro, 1972). Rapid intravenous injection of calcium (2 mg/kg) produces an immediate and prolonged rise of acid secretion in ZES but not in other ulcer patients.
3. Secretin infusion test (Isenberg, 1972). Secretin (3 U/kg per hr) usually inhibits acid secretion. In ZES, it may lead to increased acid secretion and hypergastrinemia far above basal levels. Similar observations have been reported with glucagon.

5. Therapy. If the diagnosis is certain or highly probable surgical intervention is definitely indicated. Although individual cases have been cured by the removal of a solitary non-B islet cell tumor, excision of the visible and palpable pancreas tumors is not, as a rule, sufficient in the face of the frequent multiplicity, malignancy, and ectopia of the tumors. Tumors or metastases which are overlooked give rise to recurrences. Combination with a conventional ulcer operation is also pointless, since in ZES the border cells are found throughout the stomach, and even a gastric stump will lead to hypersecretion under the influence of gastrin and thus to recurrences. If a single tumor is found on exploration of the pancreas this can be removed, and if the basal secretions measured during the operation before and after removal of the tumor show a regression and biopsy of the lymph nodes is negative, it is reasonable to end the operation there and to await developments. In the case of multiple pancreatic tumors or of recurrences, the only certain measure to be considered is total gastrectomy and perhaps resection of the tail and body of the pancreas. Zollinger has been advocating this procedure since 1958.

It is not yet known whether competitive antagonists such as antigastrin, GIP (see p. 971 and p. 975) or gastrin antibodies have potential therapeutic applications.

6. Etiology and Pathogenesis. ZES is a group of symptoms of endocrine adenomatosis. This is an autosomal dominant hereditary disease with high penetration and varying expressivity and is pleiotropic. There is associated endocrine adenomatosis in only a quarter of the cases of ZES. However, endocrine adenomatosis is found frequently among siblings, antecedents, and descendants of patients with ZES (see Chap. XVIII, p. 1007).

The symptoms of ZES have been explained by the demonstration of gastrin in the non-B islet-cell tumors of ZES (Gregory, 1960). ZES is due to the ectopic formation of gastrin, which is normally produced by the mucosa of the antrum and not by the pancreas.

Overproduction of gastrin is observed after gastric operations in which the antrum is removed from the rest of the stomach but is left attached to the pylorus (Billroth II modification after Finsterer). The gastrin production of the antrum can then no longer be inhibited by contact with the acidic contents of the stomach. This results in a maximum secretion by the remaining part of the stomach, hyperacidity, and ulcers at the anastomoses.

β) The Syndrome of Watery Diarrhea with Hypokalemia with Non-B Islet Cell Tumors (*WDHA* – *W*atery *D*iarrhea, *H*ypokalemia, *A*chylia) Syndrome, Verner-Morrison Syndrome, Pancreatogenic Cholera).

There is one variant of endocrine active non-B islet-cell adenoma which does not produce gastrin, but apparently does produce a substance which stimulates the gut to an abnormally copious secretion particularly rich in potassium and may inhibit the gastric secretion at the same time (MARKS, 1967). This substance is at present hypothetical. Glucagon, secretin (ZOLLINGER, 1968) GIP (ELIAS, 1972; Editorial 1973) or VIP (BLOOM, 1973) have been suspected. It must be postulated since the diarrhea disappears after the removal of these tumors, and gastric secretion resumes. Exclusion of HCl hypersecretion is essential for the diagnosis. Usually there is hypo- or achlorhydria. This syndrome also has the same genetic basis as endocrine adenomatosis, and these tumors may be benign or malignant.

1. Clinical Features. Massive watery diarrhea refractory to the usual treatment usually begins abruptly, and 10–20 liters are lost in up to 20 evacuations in 24 hours. The excreta are especially rich in potassium and bicarbonate, so that hypokalemia with metabolic acidosis, as well as dehydration, azotemia, and shock arise. Hypercalcemia is seen in about half the patients.

2. Therapy. Strangely, the diarrhea is considerably improved by 15–20 mg of prednisone daily. (Inhibition of kinin-activation? See p. 977). The only causal treatment is complete removal of the tumor. In contrast to ZES, a gastrectomy is useless in this syndrome. Whether other syndromes, such as that with hyperglycemia, are variations of this one has still to be confirmed or denied (LEGER, 1962; KNAPPE, 1966; RAPANT, 1965). Hypokalemia itself leads to reduced glucose tolerance.

b) Secretin

Secretin was the first gastrointestinal hormone to be found. BAYLISS and STARLING created the term "hormone" for it in 1902. It has recently been purified and its structure clarified (JORPES, 1962). It is a one-chained polypeptide of 27 amino-acid residues of the following sequence (Fig. 4): It is strikingly similar in structure to glucagon (see p. 822), also having 14 identical amino-acid residues. It has recently been successfully synthesized (MUTT, 1967).

His—Ser—Asp—Gly—Thr—Phe—Thr—Ser—Glu—Leu—Ser—Arg—Leu—Arg—Asp—Ser—Ala—Arg—Leu—Glu—Arg—Leu—Leu—Glu—Gly—Leu—Val · NH_2

Fig. 4. Porcine secretin structure formula (MUTT, 1967)

Secretin stimulates the pancreas and the bile ducts to secrete water and bicarbonate. It inhibits gastric secretion of gastrin and peristalsis, but stimulates pepsin secretion. It also causes the release of insulin (see p. 748). It has a lipolytic action and some effects on the kidney and the cardiovascular system. Secretin is formed in the mucosa of the upper small intestine and is released in response to chemical stimuli [hydrogen ions, fatty acids (MEYER, 1972)]. Its clinical importance is limited at present to the pancreas function test (DREILING, 1958). It is still too expensive for trials in the treatment of ulcers.

c) Cholecystokinin-Pancreozymin (CCK-PZ)

In 1928 IVY and OLDBERG extracted cholecystokinin from the mucosa of the upper sections of the small intestine. In 1943, HARPER and RAPER showed that an extract of the same mucosa stimulates the secretion of amylase, trypsinogen, and lipase in the pancreas. They called this gastrointestinal hormone pancreozymin. It releases the enzymes from the acinar cells and promotes enzyme synthesis (MORISSET, 1971). Later, as more purified extracts became available it was possible to state that cholecystokinin and pancreozymin were the same intestinal hormone (MUTT, 1967). The only CCK-PZ so far isolated and investigated is porcine cholecystokinin. It may be that there are several species-specific cholecystokinins. It is composed of 33 amino-acid residues and has a molecular weight of 3900. The C-terminal octapeptide of porcine CCK-PZ is

Asp–Tyr(SO_3H)–Met–Gly–Trp–Met–Asp–Phe–NH_2.

In addition to its effects on the pancreas, CCK-PZ leads to the contraction and evacuation of the gallbladder as well as to the relaxation of the sphincter of Oddi. It inhibits gastric secretion probably competitively to gastrin (BROOKS, 1970). It is released when the intestinal mucosa comes in contact with fats, peptones, and dilute hydrochloric acid.

A rather crude preparation is used for radiological and clinical diagnosis of conditions affecting the gallbladder, and in surgery of the bile ducts (JORPES, 1964).

d) Cerulein

ERSPAMER and his group detected in the skin of different species of frogs a decapeptide and

a nonapeptide, which they named cerulein and phyllocerulein respectively, with the following structure:

Pyr–Gln–Asp–Tyr(SO_3H)–Thr–Gly–Trp–Met–Asp–Phe–NH_2.

Phyllocerulein lacks –Asp– in the third position. There is a striking resemblance in chemical structure between the ceruleins and the C-terminal octapeptide of porcine cholecystokinin-pancreozymin and the C-terminal hexapeptide of the gastrin. The chemical similarity corresponds with a striking resemblance in the pharmacological effects, which are very similar or even identical to those of cholecystokinin-pancreozymin. This latter might be simply a carrier polypeptide from which a smaller hepta- or octapeptide is split off and liberated into the circulation when the flow of bile and pancreatic juice as well as intestinal movements are necessary for digestive processes. It has a very strong effect on the contraction of the gallbladder, which is estimated at least 3 times more potent than that of CCK-PZ. In doses of 5–30 ng per kg i.v. it has been used for cholecystographic studies in patients. As CCK-PZ, it has a relaxant effect on the sphincter of Oddi.

In human subjects, cerulein inhibits duodenal motility and stimulates jejunal and colonic motility and tone.

In addition, it is a potent stimulator of pancreatic secretion, not only of pancreatic juice but also of amylase and bicarbonate. In the endocrine pancreas it stimulates the release of insulin.

In the liver it stimulates the flow of hepatic bile.

It has a similar but more potent effect than gastrin in stimulating acid secretion of the stomach; however, it can also inhibit the action of gastrin, probably in a competitive way.

In addition, it seems to have a hypotensive action on the cardiovascular system, which, however, differs widely from species to species.

Untoward side effects in man occasionally consist of a sensation of heat in the face with sweating, abdominal discomfort, and nausea of short duration.

e) Hypotensive Polypeptide of Said and Mutt (VIP), Vasodilating Intestinal Polypeptide

Recently, a new intestinal hormone of polypeptide character has been extracted from porcine small intestine by SAID and MUTT (1970). The polypeptide has 28 amino-acid residues whose composition, but not the sequence, is known. In contrast to the kinins, the substance P, and other vasoactive peptides, it has no glycine or proline.

The biological activity consists mainly in arterial vasodilatation with a hypotensive effect but with an increased total cardiac output. There seems to be no direct effect on the heart muscle although a stimulation effect on respiration has been observed.

In addition, it has a hyperglycemic effect corresponding to about two-thirds of that of glucagon.

The hormone is inactivated in the liver, and thus probably has no effect on the peripheral circulation. The physiological function might be regulation of the intestinal blood flow and the blood sugar. It might have pathogenetic importance in liver cirrhosis early dumping syndrome and WDHA-syndrome (BLOOM, 1973).

f) "Gastric Inhibitory Polypeptide" (GIP)

BROWN and PEDERSON purified GIP from a crude gastrointestinal extract containing CCK-PZ which inhibits the acid secretion of the stomach. Pentagastrin, stimulated H^+ secretion and secretion induced by hypoglycemia are also inhibited. This substance could be the gastrointestinal hormone released when fat is instilled into the duodenum. Porcine GIP is a polypeptide of 43 amino-acid residues, the sequence of which has been clarified by BROWN and DRYBURGH (1971). 15 of the first 26 amino acids occur in the same position as they do in porcine glucagon and 9 of the first 26 in the same position as in porcine secretin. The calculated molecular weight is 5105.

g) Motilin

BROWN and co-workers (BROWN, 1971) isolated a polypeptide from a side fraction produced during the purification of secretin, which produced increased motor activity in the antrum and an increased pepsin output. This polypeptide was purified and is composed of 22 amino-acid residues with an N-terminal of phenylalanine. The composition of the amino-acid residues is entirely different from that in the other gastrointestinal hormones (BROWN, 1972).

h) Intestinal Glucagon (Enteroglucagon)

It seems probable that glucagon extracted from A-islet cells of the pancreas and the one extracted from the duodenal mucosa are different polypeptides (see Chap. XIII, p. 822).

2. Kinins

More recent reviews can be found in ERDÖS (1966), MELMOM (1967), WERLE (1967) and STURMER (1966).

WERLE in 1937 considered the kinins as a substance in the plasma activated by the saliva and responsible for releasing the contraction of smooth muscle. The kinins were isolated by ROCHA E SILVA in 1956, and were synthesized by BOISSONNAS in 1963. The term "kinins" is today applied to the following three biologically active polypeptides of 9 to 11 amino-acid residues.

a) Bradykinin or kinin 9.
b) Kallidin (lysyl-bradykinin, kinin 10).
c) Methionyl-lysyl-bradykinin (methyionyl-kallidin, kinin 11).

Over 70 analogues have been synthesized and their actions been examined, so that the amino-acid residues responsible for the action are known.

The kinins are the most potent known vasodilators and need neither alpha- nor beta-receptors for their action. They produce pain and cause the contraction of smooth muscle fibers (bronchospasm, increased tone and motility of the gastrointestinal tract). They increase capillary permeability and promote the migration of leukocytes.

The kinins are liberated from kininogen, an α_2-globulin which is specific to the species and is produced in the liver. They are liberated by kallikrein, a group of enzymes which occurs in the plasma, granulocytes, and different glands (salivary, tear, and sweat glands, pancreas, kidney, gut). The plasma kallikrein can be activated by various physicochemical stimuli, but usually the presence of the Hageman factor, also essential for blood coagulation, is also necessary. Trypsin, however, can also activate kallikrein, that is, convert kallikreinogen into kallikrein. Inactivators of kallikrein also appear to be normally present in the plasma.

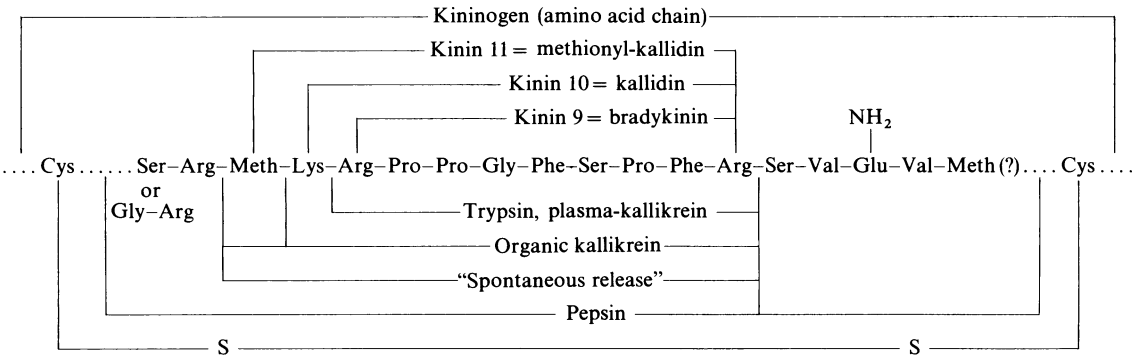


Fig. 5. Formation of kininogens and kinins released by different enzymes. (According to WERLE, 1967)

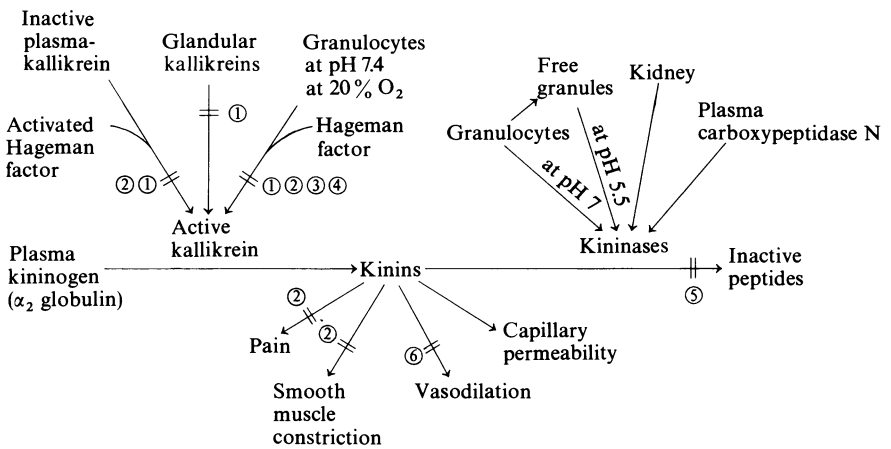


Fig. 6. Interactions of the kallikrein-kinin-kininase system, illustrating the various sources of kallikrein, the participation of Hageman factor, the sources of kininases, and the steps at which the synthesis, breakdown and effect of the kinins are influenced by pharmacologic agents. *1* glucocorticoids; *2* salicylates; *3* colchicine; *4* low oxygen, cyanide, EDTA; *5* EDTA, ε-amino caproic acid; and *6* phenothiazines = blocking.

The polypeptide trasylol, obtained from bovine lungs, inactivates kallikrein in particular, and also trypsin, chymotrypsin, and plasminogen. Apart from kallikrein, trypsin and plasmin can also liberate kinins, but to a lesser degree. It is not yet certain to what extent the β- and γ-globulins PF (the permeability factors) are similar to kallikrein or to what extent they can activate this substance (ERDÖS, 1966). Snake venom, bacterial enzymes, and pepsin can mobilize kinins. The active kinins have an exceptionally short half-life. They are inactivated within seconds by the plasma granulocytes and kidney kininases at the C-terminal end so that their concentration in the plasma normally lies below 2.5 ng/ml.

The kininase in human plasma is activated by epinephrine. Chymotrypsin inactivates bradykinin. Carboxypeptidase-B inhibits exogenous bradykinin. Kininases of the plasma are further inhibited by EDTA; ε-aminocapronic acid inactivates the carboxypeptidases (for literature see KELLERMEYER, 1968).

The physiological importance of the kinins is still unknown. They can have an effect on the change-over from the fetal to the neonatal circulation. Furthermore, they probably regulate the blood flow to the active glands and muscles. Kinins increase the blood supply to the brain, the coronary vessels, and the skin. In the kidneys, plasma flow, diuresis, sodium-potassium excretion, and the free water clearance are increased. On the other hand, the kinins are thought to cause the release of vasopressin. There is a relationship with the catecholamines which together with histamine are liberated by the kinins. Catecholamines are in turn mobilized through histamine.

The important characteristics of inflammation (rubor, calor, tumor and dolor) can be pathophysiologically explained by the action of the kinins. They are involved in endotoxin shock and in Quincke's edema, and are responsible for the flush in the carcinoid syndrome (see p. 986).

In addition, the kinins are probably involved in the pathogenesis of pancreatitis, arthritis, foreign-body granulomas, transfusion accidents, burns and in shock (for literature see KELLERMEYER, 1968).

The activation of the kinin system is related to the coagulation of the blood – both are dependent on the Hageman factor. On the other hand, immunological processes in which kinins are liberated are also connected with kinin activation.

Salicylic acid and glucocorticoids interfere with the activation of the kinins at different sites. This would partly explain their anti-inflammatory actions.

3. Renin-Angiotensin II

J. MÜLLER

a) Definition

Renin is an enzyme which can be extracted from kidney tissue. As a result of its interaction with a plasma protein, the *renin substrate* (angiotensinogen), the decapeptide *angiotensin I* is split off, which in turn is converted to the active octapeptide *angiotensin II* by a peptidase, the so-called *converting enzyme*. Angiotensin II itself is split into inactive peptide fragments by the action of different peptidases for which the collective term "*angiotensinases*" is used.

Whereas renin is exclusively or predominantly formed in the kidney, all the other reactions of angiotensin metabolism can occur in the peripheral blood. It is, however, not yet known whether in the intact organism angiotensin is produced predominantly in the kidneys, in the peripheral circulation, or in other organs under the action of stored renin. Nor do we know exactly which organs are responsible for the conversion of angiotensin I to angiotensin II and for the breakdown of angiotensin *in vivo*. From our present-day knowledge we can assume that changes in the concentration of angiotensin II in the plasma are due mainly to changes in renin secretion, whereas the remaining components of the renin-angiotensin system are usually present in excess. However, some findings have indicated that the concentration of

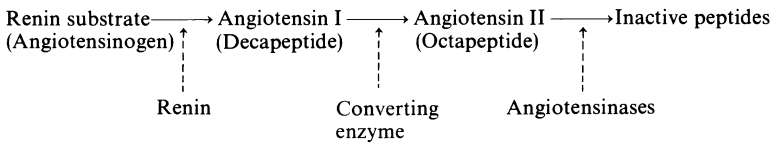


Fig. 7. Formation and degradation of angiotensin II

the renin substrate in the plasma is also under a physiological control, and it is possible that the conversion and breakdown of angiotensin are also variable.

Only angiotensin II has the properties of a hormone and is biologically active. It is not known whether the other components possess further physiological functions in addition to their effects on the production of angiotensin.

b) Biochemistry

α) Renin

The exact structure is at present still unknown. Studies with gel-filtration chromatography and ultracentrifugation indicate that the molecular weight of renin lies between 40000 and 50000. Renin is a thermolabile protein with an isoelectric point between pH 6.5 and 7.5.

From experiments with artificial substrates it can be assumed that renin splits the peptide bond between two leucine groups. The velocity with which purified renin liberates angiotensin from a standard substrate is linearly dependent on the renin concentration over a wide range and remains constant for a long period of time. Renin is also active in an electrolyte-free medium.

The species-specificity of renin activity is limited. Human, canine, rabbit and rat renin also act on a bovine substrate. By contrast, human renin does not react with porcine substrate.

Injection of renin of a foreign species leads to the formation of inactivating antibodies. Renin which is inactivated by acetylation remains antigenically active. Amazingly, it appears to be possible to provoke the formation of antibodies inactivating the endogenous renin in animals by treatment with renin of a foreign species (DEODHAR, 1964).

β) Renin Substrate

In the plasma, the renin substrate is found in the α_2-globulin fraction. SKEGGS (1963) succeeded in isolating three protein fractions from porcine plasma from which angiotensin-like peptides were split off during incubation with porcine renin. Three of these protein fractions could be purified to a high degree. These were glycoproteins with a molecular weight of about 57000 and an almost identical amino-acid composition. The organ of origin of renin substrate is still not definitely known.

According to animal experiments by CARRETERO and GROSS (1967) the renin substrate concentration in the plasma is not only dependent on the utilization but is possibly subject to a physiological regulation. Unilateral or bilateral nephrectomy, experimental renal hypertension, treatment with mineralocorticoids or excessive salt intake led to increased renin substrate levels. By contrast, low substrate concentrations were found following adrenalectomy and during sodium restriction. In humans, the plasma renin-substrate concentration is increased during pregnancy (GOULD, 1966), whereas it is decreased in the rat (CARRETERO, 1967). An increased renin substrate level in the plasma may be of clinical importance in women in whom hypertension and hyperaldosteronism occur during treatment with oral contraceptives (LARAGH, 1967).

γ) Angiotensin I and Angiotensin II

Structure:

Angiotensin I (bovine)

H_2N–Asp–Arg–Val–Tyr–Ileu–His–Pro–Phe–His–Leu–COOH

Angiotensin II (bovine)

H_2N–Asp–Arg–Val–Tyr–Ileu–His–Pro–Phe-COOH

Synthetic-β-angiotensinamide II

H_2N–β Asp(NH_2)–Arg–Val–Tyr–Val–His–Pro–Phe–COOH.

Naturally occurring angiotensin II probably has a helical structure. The amino-acid composition of human angiotensin is unknown. Porcine angiotensin II (5-valine) and bovine angiotensin II (5-isoleucine) are almost equally potent in man. The peptide most commonly used for therapeutical purposes is synthetic β-angiotensinamide II which has a prolonged effect, possibly due to increased resistance against angiotensinases.

On isolated smooth muscle, angiotensin I is about 10 to 20 times less active than angiotensin II. On the other hand, it is not certain whether angiotensin I itself has any effect on blood pressure.

The structural basis of biological activity of angiotensin II has been established by many experiments with numerous analogues. A hexapeptide with amino acids 3–8 is still weakly active. Phenylalanine must be in the terminal position and its carboxyl group must be free. A tyrosine in position 4 is obligatory (PAGE, 1962).

δ) Converting Enzyme

The chemical nature of this factor is totally unknown. Chloride ions are necessary for the conversion of angiotensin I to angiotensin II in

the plasma. The experiments of NG and VANE (1967) indicate that in the intact animal conversion does not occur diffusely in the blood but mainly during circulation in the lungs.

ε) Angiotensinases

This collective term includes several known proteolytic enzymes, such as trypsin, chymotrypsin, leucine-aminopeptidase, carboxy-peptidase, pepsin and numerous unknown enzymes from tissue and erythrocytes. The angiotensinase activity of human plasma is very high. It is, however, not sufficient to account for the short biological half-life of the pressor effect of angiotensin II. Therefore, degradation probably occurs in part extravasally, in organs such as the liver, kidney, spleen or muscles.

In the plasma, angiotensinase activity is found mainly in the α_1-globulin fraction. Aminopeptidase activity accounts for 60% of the total activity. A significant rise in the plasma angiotensinase activity has been observed by different authors in acute and chronic liver diseases (BIRON, 1967). Plasma angiotensinases are only active in the presence of calcium ions and can be inactivated by EDTA.

ζ) Methods of Determination

For methodology of plasma renin and angiotensin determinations and their normal values see Chap. VII, p. 378.

c) Physiology

α) Origin of Renin

In the mammalian organism, the major part of renin originates from the kidneys. The kidneys can secrete renin into the blood, the lymph or the urine. Renin-like substances have been isolated from the placenta, uterus and submandibular glands, but it is not definitely known whether they are identical to the renal renin.

It is generally accepted that renin is predominantly formed in the vascular pole of the glomeruli. However, it is still controversial whether renin is produced by the juxtaglomerular epitheloid cells of the afferent arterioles, or rather by the neighboring macula-densa cells of the distal renal tubules. At present, there is no definite direct evidence supporting one theory or the other. In many experimental situations the granulation of the juxtaglomerular cells is parallel to the renin content of the kidneys and it is assumed by different investigators that these granules are representative of the production or storage of renin. Under normal conditions, renin is only produced in the outer third of the renal cortex. Increased amounts of renin, however, are found in the deeper layers in renal artery stenosis.

β) Regulation of Renin Production and Secretion

At present, renin production cannot be quantitatively measured in an intact animal. Our present-day theories about the regulation of renin secretion are based on the estimation of renin concentration in the plasma or in renal tissue. Since the renin content in the kidneys changes in the same direction as the renin concentration in the plasma under most long-term experimental conditions it is assumed that production and secretion are controlled by the same regulating mechanisms.

1. Receptors and Mediators

Production and secretion of renin are influenced by renal and extrarenal factors. At present two theories of renal regulation are under discussion. They are based predominantly on morphological observations and on the assumption that the receptors of renin regulation are either identical to the renin-producing cells or in their immediate vicinity.

Baroreceptor Theory. According to this theory, renin production is mainly regulated by changes

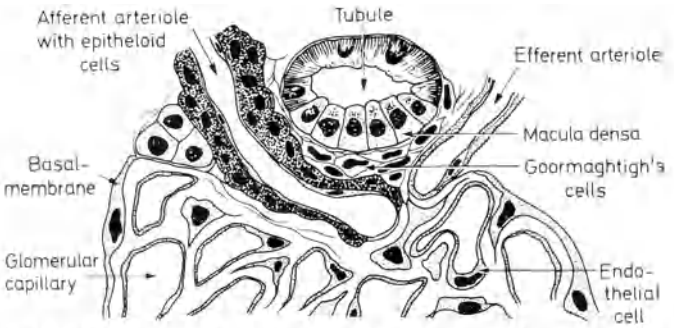


Fig. 8. Morphology of the juxtaglomerular apparatus. (From WOLFF, 1964)

in pressure. The receptors are situated in the wall of the afferent glomerular arteriole and register changes in arterial blood pressure, in vascular tone or in interstitial renal pressure.

Macula-Densa Theory. According to the opinion of other authors, renin production changes predominantly in response to changes in the composition of the urine in the distal tubule. According to this theory, the macula densa cells are chemoreceptors registering changes in sodium flux.

Neural Regulation. It is certain that renin production is influenced by the sympathetic nervous system. However, we do not know whether this is by direct stimulation of the renin-producing cells or by indirect amplification of the stimuli acting on the two postulated intrarenal receptors. The juxtaglomerular apparatus is richly supplied with non-myelinated nervous fibers. Denervation of the kidney leads to a fall in its renin content. Infusion of catecholamines, tyramine or dimethylphenylpiperazine into the renal arteries induces increased renin secretion.

Hormonal Regulation. Angiotensin exerts a negative feedback effect upon renin production. This suppression of renin production is independent of the plasma aldosterone level or of renal arterial blood pressure. Infusion of aldosterone into the renal artery of a sodium-deficient animal does not suppress renin secretion. It is uncertain whether hormones other than catecholamines and angiotensin can directly influence renin production.

2. Variations in Renin Secretion under Physiological and Pathological Conditions

Biological Rhythms. In normal humans, there is a circadian rhythm of plasma renin concentration which is independent of posture and food intake. In recumbent subjects, the highest values were found between 2 and 8 a.m., the lowest values between noon and 6 p.m. (GORDON, 1966).

Upright posture leads to a steep increase in plasma renin concentration, but the increment is larger in the morning than in the afternoon. The rise of renin can be suppressed by bandaging the lower half of the body.

In normal women, plasma renin concentration increases during the luteal phase of the menstrual cycle.

Sodium Balance. Acute or chronic sodium deficits due to a sodium-deficient diet, saliuretics, tubular sodium loss, Addison's disease or adrenalectomy lead to a marked increase in plasma renin concentration, excessive oral or parenteral sodium intake or treatment with mineralocorticoids to decreases in renin concentration.

Alterations of Circulating Blood Volume. Hemorrhage or hypotension lead to an increased secretion of renin whatever the etiology. An increase in renin production in thirst and in edematous diseases (cirrhosis of the liver, nephrosis) is probably also due to a decrease in circulating blood volume. Elevated renin concentrations are not usually found in patients with untreated congestive heart failure.

Hypertension. Normal or low plasma renin values are found in benign essential hypertension. In renal or malignant hypertension, the plasma renin concentration is often but not always elevated. There is no correlation between hypertension and the renin concentration; on the other hand, there is a reversed correlation between the sodium concentration and the renin concentration in the plasma. However, it is not certain whether the low plasma sodium concentrations found in some patients with renal hypertension are the cause or a consequence of the elevated renin level.

Pregnancy. Plasma renin concentrations are within the normal range in approximately 50 percent of normal women throughout pregnancy. In the others, plasma renin concentration increases during early pregnancy, stays at a high level until delivery and then decreases rapidly to normal values. The renin may be formed partly by the placenta. Normal or decreased renin values are observed in preeclampsia.

γ) Physiological and Pharmacological Effects of Angiotensin II

1. Effects on the Circulation

Blood Vessels. Angiotensin II has a marked vasoconstrictive effect mainly on the precapillary vessels, whereas its constrictive effect on the postcapillary vessels and the veins is only weak. Its predominant effect is on the vessels of the skin, of the splanchnic area and of the kidney, resulting in an acute reduction of the blood supply of these organs. Its effects on the vessels of skeletal muscles and on cerebral, coronary and pulmonary circulation are considerably less pronounced.

Heart. In vitro, angiotensin exerts a positive inotropic effect on the cardiac muscle (KOCH-

WESER, 1964). However, it is very questionable whether it exerts the same activity *in vivo*.

Blood Pressure. Angiotensin II is one of the most potent hypertensive agents. On a molar basis, it is 40 times more active than noradrenaline. Even a dose of 2 ng/kg/min can lead to a considerable rise in blood pressure. After an intravenous injection of angiotensin II the blood pressure reaches a maximum within one minute and becomes normal again within five minutes without a secondary fall below normal. Since the pressor activity of angiotensin is still demonstrable after adrenalectomy, sympathectomy, and cordotomy, as well as during treatment with atropine, ganglion blockers and adrenergic blockers, it is assumed that it acts directly on the vascular muscles. However, a stimulatory effect on the symphathetic nervous system may contribute to its overall effect on blood pressure.

Adrenal Medulla. Angiotensin acts directly on the chromaffin cells and leads to the release of catecholamines.

2. Effects on the Kidneys

Angiotensin causes an acute decrease in the renal blood supply and thus decreases the glomerular filtration rate. As a result, there is a decrease in water and electrolyte excretion. This antidiuretic effect has been observed mainly in humans and dogs. On the other hand, angiotensin inhibits sodium reabsorption in the distal tubules and thus leads to sodium and water diuresis. This diuretic action has been observed mainly in rabbits and rats. However, it is also demonstrable in man under certain conditions. Certain forms of renal hypertension lead to sodium loss. Administration of angiotensin in patients with cirrhosis of the liver and ascites leads to a marked salt-and-water diuresis.

3. Effects on the Adrenal Cortex

Angiotensin in small doses specifically stimulates the biosynthesis and secretion of aldosterone. In many cases of so-called secondary aldosteronism, an elevation in aldosterone secretion rate appears to be directly related to an increased renin production. Alterations of aldosterone secretion in response to alterations in sodium balance are at least partly mediated by the renin-angiotensin system.

4. Effects on Smooth Muscles

Angiotensin not only provokes contractions of vascular smooth muscles, but has a similar effect on the smooth muscles of the gut, uterus, gall bladder, seminal vesicles and ureters. These effects, however, are at least partly mediated by the autonomic nervous system. Thus, the effect of angiotensin on guinea-pig ileum can be partly suppressed by atropine.

d) Hyperfunctional and Hypofunctional Clinical Syndromes of the Renin-Angiotensin-System

Recently, 6 cases of renin-producing renal tumors, "hemangiopericytomas", originating from the juxta glomerular cells have been observed. The patients had hypertension and in a late phase hypokalemia as patients with primary aldosteronism. However, renin activity in the serum was high and especially elevated in the venous plasma from the affected kidney. After operations, the patients were cured, and renin-like enzyme could be extracted from the tumors. Also other, more common tumors as Wilms's tumor, may occasionally be associated with "primary reninism" and hypertension in children (Leading article, 1973; CONN, 1972).

On the other hand, isolated hypoaldosteronism with recurrent weakness and blurring of consciousness with hyperkalemia can be due not only to blocked biosynthesis of aldosterone, but apparently also to a deficiency of renin. Infusion of angiotensin II corrects plasma aldosterone, and the patients are successfully treated with fludrocortisone (Leading article, 1973; BROWN, 1973).

4. Substance P

In 1931 EULER and GADDUM described a new type of substance, probably of peptide structure, in extracts of the brain and gut. This substance, even in the smallest amounts, stimulated smooth muscle, salivary secretion and caused a fall in blood pressure.

This substance P, has now been purified, it's amino-acid sequence clarified; and recently it has been synthesized (CHANG, 1971; TREGEAR, 1971). It is a one-chain hendecapeptide.

Substance P is found in the intestinal tract and in the nervous system of man and of all the vertebrates examined (HAEFELY, 1962; LEMBECK, 1962; ZETLER, 1963).

Substance P causes most of the organs with smooth muscle to contract and causes the blood pressure to fall as a result of peripheral vasodilatation (PERNOW, 1953). Based on the grounds of occurrence of substance P in the central nervous system and on certain actions of poorly purified preparations, it has been postulated that the polypeptide has some function as an inhibitory transmitter substance at cen-

tral synapses (LEMBECK, 1962; ZETLER, 1963). Experiments with highly purified preparations, however, did not provide evidence of the specific neurotropic property of the substance (HAEFELY, 1962; BAILE, 1967). The assessment is, however, encumbered by the exceptional instability of the purified substance. An enhancement of intestinal peristalsis seems to be its most probable physiological action.

The hendecapeptides *Eledoisin*, extracted from Salivary glands of cephalopods and *Physalaemin*, isolated from amphibian skin, have a similar constitution and similar effects.

5. VEM and VDM (Vasoexcitor and Vasodepressor Substances)

In 1945, SHORR, MAZUR, and BAEZ discovered the vasoactive substances VEM and VDM during their studies of experimental shock. There is still a difference of opinion about whether these substances should be termed "tissue hormones", and this can only be settled after the physiological importance of these substances has been finally determined.

VDM is formed in the liver, spleen, and skeletal musculature. VEM is formed by the kidneys. The VDM produced in the liver has been identified as the Fe-containing protein, ferritin. Its hypotensive action is bound with its reduced sulfhydryl-ferro form. It loses its effect in the oxidized state. This is thus dependent on the prevailing oxygen tension. Apart from this, the adrenocortical hormones, protein intake, and ascorbic acid influence its activity. Ferritin reduces the reactivity of the capillaries to adrenaline, especially in the splanchnic area. In addition, it is thought to have an antidiuretic effect via the release of vasopressin and also has a function in the storage and liberation of iron. Very little is known about the nature of the VDM of muscular origin and of the hypertensive VEM.

6. Parotin

It is still uncertain whether a protein extracted from the glandula parotis and glandula submaxillaris, which stimulates calcification of the connective tissue in certain mammals, especially the teeth, lowers the serum calcium, and favors leukocytosis, is a hormone or not (ITO, 1960). Further, a protein NGF has been extracted from submaxillary glands of mice, which stimulates the growth of sympathicus nerve cells in the mammal (COHEN, 1960; LEVI-MONTALCINI, 1968). Other factors from the same source are said to stimulate the erythropoietin and the granulocytes (LEVI-MONTALCINI, 1968).

C. Glycoprotein: Erythropoietin

In 1906 CARNOT and DE FLANDRE observed that serum of anemic rabbits promoted the production of erythrocytes in the normal animal. This substance, now named "erythropoietin", has received intensive attention only in the past 20 years.

Erythropoietin is a glycoprotein, or a polypeptide with carrier protein. It is probably an alpha-globulin with a molecular weight of 28000–33000, containing neuraminic acid, 7–8% hexoses, hexosamine and sialinic acid. Despite a 50000-fold concentration from serum of anemic sheep, especially by means of ionic exchange chromatography, the hormone has still not been produced in purified form. In spite of weak antigenic properties, antibodies are retained which neutralize erythropoietin *in vivo* and *in vitro*. The antigen-antibody reactions are not species-specific (SCHOOLEY, 1962).

There might be a renal erythropoietic factor which converts a substance formed in the liver to active erythropoietin.

An insufficient oxygen supply is an adequate stimulus for the formation or release of erythropoietin. Thus, hypoxia and severe anemia can cause a rise in the plasma content, whereas the erythropoietin level falls with over-transfusion or with the return to sea level after adaptation to heights. The daily production, according to the urinary excretion, is estimated to be 4.0 U (WHO Standard B) with variations of 1.2–9.5 U (ADAMSON, 1966).

Hormones of the pituitary, gonads, thyroid, adrenal cortex and medulla are nonspecific stimulators of erythropoiesis. Androgens promote the formation of erythropoietin directly, whereas estrogens inhibit it.

Erythropoietin acts on the parent cells of the bone marrow and stimulates their differentiation into erythroblasts. It also seems to govern the rate of hemoglobin synthesis in the erythroblasts already differentiated. Further Cobalt ions increase the formation of erythropoietin. The erythropoietin content of the blood falls after nephrectomy and can no longer be stimulated by cobalt. Erythropoietin is formed mainly in the renal tissues, probably in the juxtaglomerular apparatus. It is possible that a small amount is also produced extra-renally.

Erythropoietin in the plasma is increased in all sufficiently severe cases of anemia. Erythropoietin is excreted in the urine in amounts definitely dependent on the severity of the anemia.

The erythropoietin is demonstrated by measurement of the ^{59}Fe incorporated into the erythrocytes in the overtransfused mouse after

administration of the serum or urine extract to be examined.

Renal tumors which give rise to polyglobulism contain and produce more erythropoietin. Erythropoietin is also accumulated in the fluid of renal cysts, in the cysts of cystic kidneys, and in hydronephrosis. This can lead to polycythemia probably secondary to partial ischemia of kidney tissue (ADAMSON, 1968). There is a series of other tumors known to lead to secondary polyglobulism.

D. Amines

1. Serotonin (5-hydroxytryptamine, enteramine)

Serotonin is formed in the enterochromaffin or argentaffin cells of the gastrointestinal tract as well as in the central nervous system. Mast cells form serotonin in the rat and mouse, but not in man. Serotonin probably acts as a local hormone (parakrinial), and can exert distant effects by means of the bloodstream in which it is transported predominantly by the thrombocytes. The blood platelets can concentrate serotonin from the plasma by more than a thousand fold, the uptake capacity being dependant of an intact energy metabolism with a normal ATP content. The abundance of serotonin in the spleen is probably due to the accumulation of thrombocytes. In the central nervous system, the hypothalamus in particular contains large amounts of serotonin and catecholamines, whereas the pineal body also forms the serotonin derivative, melatonin (see Chap. IV, p. 73).

a) Biochemistry

The biosynthesis of serotonin from tryptophane occurs by processes similar to the synthesis of the catecholamines from phenylalanine. Some of the same enzymes are employed. The dopa-decarboxylase also decarboxylates hydroxytryptophane to hydroxytryptamine. 5-Hydroxylation by tryptophane-hydroxylase is the first step. Under normal conditions, about 1% of the tryptophane taken in with the food is converted to serotonin. This percentage can rise to 60% under pathologic conditions such as the carcinoid syndrome.

Breakdown occurs in the first place through the actions of monoamine oxidase, which converts the serotonin into hydroxy-indole acetic aldehyde. Monoamine oxidase is also involved in the breakdown of a series of other amines but shows a preference for serotonin. 5-Hydroxy-indole acetic acid is formed from the aldehyde through an aldehyde dehydrase and is then excreted through the kidneys. The individual

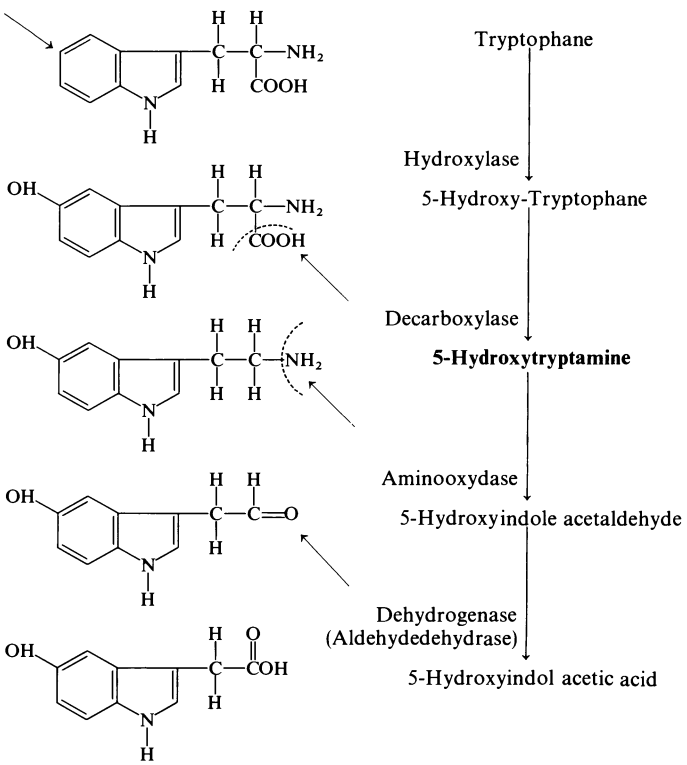


Fig. 9. Biosynthesis and metabolism of 5-hydroxytryptamine (serotonin). (From CERLETTI, 1958)

Table 1. Inhibitors of formation and degradation, releasing factors and antagonists of serotonin. (According to LEMBECK, 1962)

Inhibitory substances	Enzymes		Liberators	Antagonists
		Tryptophane		
		↓		
		Hydroxytryptophane		
α-Methyl-Dopa, Phenyl acetic acid	Decarboxylase	→		
		Hydroxytryptamine (Serotonin)	Reserpine *et al.*	LSD, Deseril, Medomin, etc.
Iproniacide, Niamide *et al.*	Monoamine oxidase	→		
		Hydroxyindole acetic acid		

stages of the building up and breaking down processes can be inhibited by enzyme inhibitors. This, however, is only of theoretical interest and cannot be used therapeutically (see p. 970).

b) Physiology

In 1966 a handbook of 724 pages on hydroxytryptamine was published. Although the pharmacological research of serotonin is very advanced, little is known about its physiology. Reserpine and similar compounds liberate serotonin from the brain and the thrombocytes, yet they have only a slightly similar effect on the enterochromaffin cells of the intestinal tract. Almost nothing is known about the physiological releasing mechanism. Adrenaline and noradrenaline can liberate serotonin. The only probable physiological action of serotonin known today is the increase in the motility of the gut. i.e., a fall in the stimulus threshold for mechanical influences acting on the Meissner's plexus to release peristaltic waves.

It is not known whether serotonin has any effect on blood coagulation through the destruction of the thrombocytes which is associated with the liberation of large amounts of serotonin. Depletion of serotonin stores through large amounts of reserpine does not produce a prolonged bleeding time. It is possible that in the case of a pulmonary embolus, the serotonin liberated through the destruction of platelets increases the pressure in the pulmonary circulation slightly. Only unphysiologically high doses of serotonin have an antidiuretic action, probably because they release vasopressin. It has no effect in diabetes insipidus (REBER, 1956). The renal blood supply is not influenced by physiological doses. The physiological significance of serotonin in the brain is still unexplained. Depletion of the stores by means of reserpine or para-chlorophenylalanine leads to depression, insomnia and hallucinations. The insomnia can be counteracted in animal experiments by the administration of 5-hydroxytryptophane (JOUVET, 1967). The concentration of serotonin in the brain varies during day and night, so that serotonin has also been called "somnotonin" (KOELLA, 1970). The hallucinogenic lysergic acid diethylamide is an antagonist to serotonin in various peripheral organs. However, it has not yet been proven that its actions are due to the inhibition of serotonin. Iproniazide, a monoamine oxidase inhibitor, results in the accumulation of serotonin in the brain, and is described as a psychoenergetic agent by the pharmacologists. It inhibits the breakdown of the catecholamines as well as of serotonin.

c) Carcinoid Syndrome

Today it is doubtful whether the carcinoid syndrome is due entirely to the over-production of serotonin. However, there is, almost without exception, increased production of serotonin in this syndrome. Over-production of serotonin is probably responsible for individual symptoms if not the cause of the syndrome. Carcinoid syndrome is dealt with here for these reasons as a hyperfunctional syndrome of the tissue hormone, serotonin.

α) Morbid Anatomy

The carcinoid syndrome has two main forms (Table 2); the pseudocarcinoid syndromes, i.e. groups of symptoms simulating carcinoid syn-

Table 2. Different forms of carcinoid syndrome

	Tumor	Clinical symptoms	Biochemistry
1. Typical carcinoid syndrome	Carcinoid	Typical	Typical
2. Atypical carcinoid syndrome	a) Carcinoid b) Noncarcinoid	Atypical Typical	Atypical Typical

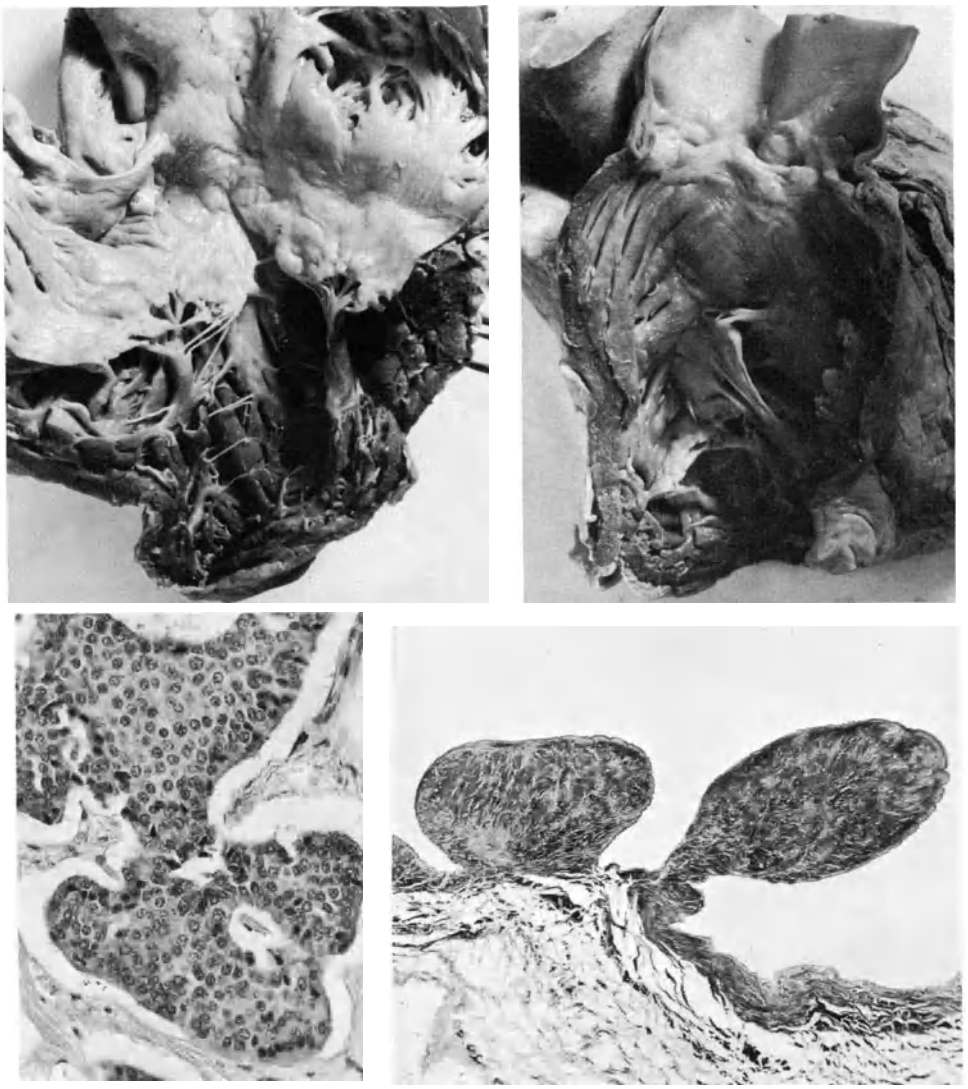


Fig. 10. Carcinoid syndrome. S 1597/53, 76-year-old male. Upper left: Fibrous thickening of the tricuspid valve and of the endocardium in the right atrium. Upper right: Chronic fibrous endocarditis of the pulmonary valves with pulmonary stenosis. Lower left: Hepatic metastasis of a carcinoid of the ileum. Lower right: S 726/56, 65-year-old male with metastasizing carcinoid. Polyp-like fibrous thickening of the endocardium in the right atrium

drome, must be differentiated from these two forms.

Carcinoids. Carcinoids are usually situated in the gastrointestinal tract, primarily in the thin bowel, in the appendix and less often in the stomach, the colon, or in Meckel's diverticulum. Carcinoids, however, also occur in the bronchial tree or in teratomas of the ovaries or testicles. Carcinoids of the gastrointestinal tract derive from argentaffin or enterochromaffin cells characterized by their affinity to silver salts which they reduce without reducing agents to metallic silver and form granular black precipitates in the cytoplasma of these cells. A part of the tumor is, however, only argyrophil, i.e., the granula are made black by silver salts only if a reducing agent is added. The argentaffinity is very labile and can disappear within two hours, so negative findings are to be cautiously considered. There is no parallel between the frequency of the carcinoids in the different parts of the gut and the number of the argentaffin cells in the corresponding parts. Primary tumors of the gastrointestinal tract are usually small, the size of a cherry, and occasionally multiple. The metastases occur especially in the mesenterial lymph nodes and in the liver. They can also occur in more distant parts. The full-blown carcinoid syndrome usually only occurs if meta-

stases in the liver have developed. Mass and extension of the metastases do not parallel the intensity of the syndrome. Carcinoids outside the portal system, e.g., carcinoids in teratomas of ovaries and testicles, can produce a full carcinoid syndrome without metastases. The carcinoids of the bronchial tree produce the syndrome only if liver metastases occur. The carcinoids of the stomach produce especially atypical syndromes (see p. 990). This type seems to produce predominantly bone metastases.

Other Tumors with Carcinoid Syndrome. Anaplastic, particularly oat-cell bronchial carcinoma, can produce the carcinoid syndrome without typical carcinoids. Also, medullary carcinoma of the thyroid, tumors of the pancreas, and especially of the islets, as well as other tumors can produce the syndrome.

Cardiac Changes. The most impressive secondary symptoms are the changes in the heart. Metastasizing thin-bowel carcinoids, changes of the tricuspid and pulmonary valves with pulmonary stenosis form a typical triad (ISLER and HEDINGER, 1953). The changes in the endocardium are due to a thickening of the tricuspid and pulmonary valves with a strange frosting-like mass which spreads over the surrounding parts of the endocardium of the right atrium, right ventricle, and the pulmonary artery (Fig. 10). The thickening consists of a tissue rather rich in cells with a basophilic and metachromatic ground substance spread over the endocardium. Occasionally, the valves of the left heart can also be attacked, especially if there is a shunt between the right and the left heart, for instance if there is an open foramen ovale.

Vascular Changes. In the regions where the flush occurs, there are enormous dilatations of the subepidermal capillaries (angioma telangiectaticum – SCHOLTE, Steiner-Voerner syndrome – PARKES-WEBER) with edema and basophilic infiltration of the surrounding collagen tissue. After removal of the tumor these changes may regress.

Other Findings. Besides the flush and its sequelae, a kind of pellegra may occur which disappears after removal of the tumor or after treatment with niacin. Occasionally, a thickening of the collagen tissue of the pelvis with a ring-like wall around the bladder and the rectum has been observed. There are no significant inflammatory infiltrates. It is possible that there is fibrosis as a sequel to accumulation of agents which act on the connective tissues and are secreted by the metastasizing carcinoid into the abdominal cavity.

β) Frequency and Incidence

Asymptomatic carcinoids are found relatively often. In 0.65% of all necropsies there are small carcinoids in the gastrointestinal tract. Carcinoids are found in about 0.2–0.5% of the surgically removed appendices. Classic carcinoid syndromes are, however, relatively rare. WALDENSTRÖM (1962) in Malmö observed 6 cases in a population of about 200000 persons within a period of 10 years. In the same period, there were three cases of pheochromocytoma and only one active insuloma.

Atypical carcinoid syndromes with simultaneous hyperinsulinism, ACTH overproduction, prostaglandin secretion, histamine release, etc. are rarities. Carcinoid syndromes due to other types of tumors or carcinoids in endocrine adenomatosis seldom occur. The relative frequency of different localizations of carcinoids is reviewed in Table 3.

Table 3. Localization and incidence of carcinoids

Localization	Incidence (%)	Argentaffinity	
		Carcinoid	Normal mucosa
Bronchial tree	1	Rarely	(+)
Stomach	3	Rarely	++++
Pancreas	Rarely	Often	(+)
Duodenum	2[a]	Mostly	+++++
Jenunum / Ileum	33[a]	Mostly	++
Meckel's diverticulum	1	Often	++
Appendix	46	Often	++
Colon	2	Not always	+
Rectum	1	Rarely	+++
Bile ducts	0.2	Rarely	+
Hamartomas	Rarely	Variable	
Teratomas	Rarely	Variable	

[a] Craniocaudal increase in incidence.

The statistics published by MOERTEL (1961) show a male preponderance of approximately 2:1. The patients are mostly between 50 and 60 years old. The syndrome has, however, also been found in juveniles and elderly people.

γ) Clinical Features

The clinical illness is dominated by the following four cardinal symptoms:

1. Skin symptoms: flush, constant cyanosis, telangiectases.
2. Gastrointestinal symptoms: colic, diarrhea, obstruction.

	Symptom	Incidence	%→
Skin	Flush	129	93.5
	Constant cyanosis	25	18.1
	Telangiectasias	35	25.2
	Pellagra-like dermatosis	9	6.5
Respiratory system	Asthma	26	18.9
Gastrointestinal tract	Diarrhea	107	77.5
	Colic-like abdominal pain	70	50.7
	Ileus or subileus	21	15.2
Heart	Endocardial fibrosis in the right ventricle (autoptically) or valvular alteration proven by heart catheterisation	55	39.9
	Endocardial fibrosis of the left ventricle (only detected by necropsy)	18	13.1
Water balance	Oliguria or edema (no signs of cardiac diseases)	26	18.9
Articulations	Joint pains, "Arthritis"	10	7.3
Tumor	Palpable tumor of the liver	86	62.4
Body weight	Weight loss	65	47.1

Fig. 11. Type and incidence of symptoms in typical carcinoid syndrome in 138 patients. (According to KÄHLER, 1967)

3. Respiratory symptoms: bronchial asthma, tachypnea, hyperpnea.

4. Cardiac symptoms: pulmonary stenosis, tricuspid insufficiency, right-sided failure.

The frequency of the main and minor symptoms is given in Fig. 11.

The symptoms start slowly and the course may be drawn out over years or even decades since the tumors grow slowly and are relatively benign in spite of metastases. On average, however, the illness lasts for 2–4 years, and the long-term prognosis is poor with very few exceptions.

1. Skin Symptoms. The most characteristic and pathognomonic symptom in the typical course of the disorder is the flush: a striking reddening of the face, consisting of large spots or patches, which may change into cyanosis and may be associated with a sensation of heat. The duration of an attack and the frequency can vary greatly. As a rule, the attacks last for a few minutes but may last for hours. At the beginning they arise monthly or weekly. Later, they can occur several times a day. The flush develops spontaneously at any time, but it is especially frequent on wakening. It can, however, be precipitated by physical exertion, excitement, intake of food, especially of alcohol, and by mechanical pressure on the tumor or on metastases. In addition to the unpleasant sensation of heat, the patients suffer from congestion in the head and sometimes from nausea and vomiting. They may experience a burning in the areas of skin involved. Itching seldom arises. The flush goes through three stages. First the precapillary arterioles become dilated. Cheeks and nose become brick red, and the reddening spreads to the neck, thorax and perhaps the extremities. The conjunctivas are injected. The skin then becomes purplish red. At the same time edematous swelling arises, especially in the lips and lids. The skin is hot. In the subsiding phases, patchy cyanosis develops – the arterioles are constricted and the capillaries dilated. The skin is then cold. The cardiac output is first reduced, then increased. Tachycardia develops, with slightly elevated diastolic and systolic pressures. Finally, hypotension arises, and collapse may ensue.

Apart from the flush during attacks, persistent reddening of the face may develop, and finally, constant cyanosis due to local hemostasis in the dilated capillaries. The telangiectases on the nose and cheeks are characteristic. They may disappear after the tumor has been removed. Finally, pellagra-like changes become noticeable in the skin as goldenbrown pigmentation in the

face and on the back. Hyperkeratosis may be associated with stomatitis, glossitis and vulvitis.

2. Gastrointestinal Symptoms. These arise early in the form of irregular watery diarrhea and colic-like attacks of pain. They may occur together or separately. Loud borborygmi are characteristic. Colic and diarrhea occur either occasionally or as often as 20 times daily. The feces may contain undigested food, and radiological examination reveals exceptionally accelerated motility of the gut. Occasionally steatorrhea may develop.

Obstruction, sub-ileus, and ileus are due to the primary tumor or the metastases.

Finally, in over 50 percent of cases, a tumor is palpable in the liver.

Diarrhea and colic may arise with the flush or independently.

3. Respiratory Symptoms. As a rule, the flush is associated with tachypnea and hyperpnea, which is usually accompanied also by bronchial spasm with typical bronchial asthma. Occasionally the asthmatic symptoms may precede the typical carcinoid symptoms. The asthma varies from breathing with mild stridor to extremely severe attacks with asphyxia.

4. Cardiac Symptoms. Endocardial fibrosis involves predominantly the right side of the heart. Changes in the left side of the heart have only been found at autopsy and almost exclusively with pulmonary carcinoid or with intracardial shunts. The cardiac changes are a late symptom, and they are irreversible. In rare cases the pericardium is slightly affected by fibrosis as well as the endocardium. Half the patients with cardiac involvement die of cardiac failure; pulmonary valvular stenosis, occasionally combined with mild pulmonary insufficiency and all the corresponding typical auscultatory findings, dominates the clinical picture. The values obtained by cardiac catheterization are also typical. The ECG shows right ventricular overload and damage. The X-ray may reveal a protruding pulmonary arch. The right ventricle does not become enlarged until cardiac failure develops. Tricuspid insufficiency usually arises only when the pulmonary stenosis leads to increased pressure. The right side of the heart soon becomes insufficient and fails. Tricuspid insufficiency can be recognized from the venous and hepatic pulsations.

5. Other Symptoms. Disturbances in water balance – oliguria, edema, and occasionally ascites and pleural effusions – are less frequent symptoms. They may also occur in the absence of cardiac failure. Their development cannot be readily explained. Serotonin is thought to increase the venous pressure and capillary permeability.

Joint pains in small and large joints, occasionally with slight inflammatory signs, do not necessarily correspond to true arthritis. Duodenal ulcers are thought to occur frequently.

Cases of bronchial carcinoid with hypoglycemia and increased immunoreactive insulin in the serum as well as in the tumor have been observed (SHAMES, 1968).

6. Psyche. Critical investigation of patients with carcinoid syndrome has not revealed any psychopathologic manifestations which can be directly related to 5-hydroxytryptamine. Acute or chronic over-administration of serotonin does not have any immediate effect (SCHNEIDER, 1957; KIND, 1957).

In addition to the full-blown presentation which is easy to diagnose, individual organic symptoms may completely dominate the picture. Thus a tumor form, an enteral form, a cardiovascular form, and a pulmonary form can be differentiated (HEDINGER, 1962).

δ) Pathogenesis

Not every carcinoid, and not even every carcinoid with liver metastases, results in carcinoid syndrome. Conversely, in rare cases, the full picture of the syndrome may be found in the absence of the morphological correlate of a carcinoid tumor.

Carcinoids are tumors of the enterochromaffin or argentaffin cells which form the tissue hormone serotonin. The tumors contain relatively large amounts of serotonin while dopadecarboxylase activity is high and amino oxidase activity low, so that the serotonin pool in the carcinoid patient can be estimated to be as much as several grams (ZIEGLER, 1967). The level of serotonin in the blood is elevated almost without exception, and increased amounts of 5-hydroxy indole acetic acid, the breakdown product of serotonin, are excreted in the urine. A hyperfunctional syndrome of serotonin was formerly thought to be recognized in the carcinoid syndrome, since serotonin acts on the gut, dilates the capillaries and produces edema and bronchial spasm. Supporting this view is the fact that pressure on the tumor or on the metastases can cause an attack. On the other hand, however, one finds that during the flush, the serotonin in the blood is not raised, and that injections of serotonin produce certain vascular reactions but are hardly capable of releasing a typical flush.

In addition, despite all efforts, cardiac changes have not been successfully produced in animal experiments by the chronic administration of serotonin which would correspond to the carcinoid syndrome. Serotonin over-production is probably responsible for the diarrhea at the most.

Tumor metastases in the liver, in contrast to healthy liver tissue, contain considerable amounts of kallikreinogen, or kallikrein, the enzyme which liberates the kinins, particularly kallidin or lysyl-bradykinin, from the kininogen in the plasma. Bradykinin releases the typical flush in both the healthy subject and the carcinoid patient. At the same time it causes bronchospasm and intestinal contractions. As a rule, however, the concentration of bradykinin in the hepatic vein is not always elevated during the flush.

The catecholamines, adrenaline and noradrenaline, can also produce a typical flush. The carcinoid tumors, especially metastases, liberate kallikrein under the influence of catecholamines, which then leads to a rise in the bradykinin in the blood. On the other hand, serotonin can also liberate catecholamines and vice versa. It is probable that all three substances are involved in the flush, the diarrhea, and the asthma to some extent. It can be concluded that the diarrhea is due predominantly to serotonin, since the serotonin antagonist, methysergide, causes only the diarrhea to cease. The flush can be counteracted successfully with glucocorticoids. It is known that glucocorticoids inhibit the activity of kallikrein (see p. 977). The pathogenesis of the cardiac symptoms has not yet been explained. It is likely that they are produced by a substance found in the vena cava and only rarely contained in the pulmonary veins. It is generally assumed that both serotonin and bradykinin are found in higher concentrations in the pulmonary artery than in the pulmonary veins. However, changes in the left side of the heart may also occur in the absence of pulmonary carcinoids, metastases, or of a right-left shunt. Bradykinin increases endothelial permeability. It has yet to be proven that bradykinin or similar substances cause endocardosis. The changes in the heart are not related in any way to the duration of the illness or to the size of the liver metastasis (ROBERTS, 1964).

The predisposition to edema and oliguria may be due to serotonin. The pathogenesis of the joint pain is not clear. The pellagra-like cutaneous manifestations and pigmentations are probably the results of a nicotinic acid deficiency. This deficiency is probably due to the removal of tryptophane by the carcinoid tumors so that the basic substance is not available in adequate amounts for nutrition.

ε) Laboratory Diagnostic Tests

Routine laboratory investigations such as ESR, blood picture and urine analysis generally yield normal results. Occasionally thrombocytosis is found. There is hypoproteinemia in about 25 percent of cases.

The diagnosis is confirmed by the demonstration of a definitely increased content of 5-hydroxy indole acetic acid in the 24-hour urine. This substance can be estimated colorimetrically after suitable extraction and purifying processes (UDENFRIEND, 1955). The qualitative and semiquantitative urinary tests are not reliable. Conditions necessary for the test are the withdrawal of all possible drugs, especially those of the phenothiazine group, and the elimination of fruit with a high serotonin content, such as bananas, walnuts, and pineapples from the diet, before collection of the 24-hour urine.

Values of 2–8 mg are considered normal. Values above 15 mg/24 h are certainly pathologic. Values of 50–300 mg/24 h are found in carcinoid syndrome. A distinctly elevated value is evidence of carcinoid syndrome. Normal values, however, do not exclude a carcinoid. Serotonin may be released intermittently, and the amount of 5-hydroxy indole acetic acid in the urine may vary from one day to the next. When there is clinical suspicion of a carcinoid and the values are normal, a provocative test with reserpine is indicated. This test proves that a carcinoid is present when the result is positive but does not exclude a carcinoid when the result is negative. 2–5 mg reserpine is given by i.m. injection, and the 5-hydroxy indole acetic acid excreted in the 24-hour urine is measured for 2–3 days. A rise of over 15 mg excretion is required for a positive result. Slight increases occur in normal individuals. If the test gives a negative result with this dose and the reserpine is well tolerated the test can be repeated with 5–10 mg reserpine (LANGEMANN, 1955; KÄHLER, 1967).

Attempted precipitation of a flush by administration of 1–10μg adrenaline intravenously is a useful clinical test.

ζ) Differential Diagnosis

The carcinoid flush must be differentiated from menopausal hot flushes which are not, however, so severe and are not associated with cyanosis and the unpleasant concomitant manifestations. The swelling of the lids and mouth may suggest Quincke's edema. The histamine-induced flush in mastocytosis, i.e., urticaria pigmentosa, is bright red, lasts longer, and results in pigmentation. Finally, the emotional rubeosis of vege-

tatively labile young people may occasionally come into the differential diagnosis.

If the diarrhea is not accompanied by a flush, all types of spasmodic diarrhea especially those due to endocrine factors, must be considered in the differential diagnosis. In contrast to bronchial asthma, bronchial spasm in the carcinoid syndrome is always associated with tachypnea and hyperpnea. The cardiac symptoms of the carcinoid syndrome arise late, and acquired pulmonary stenosis in later life, especially in combination with tricuspid insufficiency, is almost pathognomonic.

η) Therapy
(KABAKOW, 1959; MENGEL, 1965; KÄHLER, 1967)

When the carcinoid syndrome becomes manifest, metastases are almost always already present, with the sole exception of the rare gonadal carcinoid. Curative treatment by surgery is therefore only possible in a few cases. Surgical intervention is, however, vitally indicated in cases of intestinal obstruction and as a palliative measure in the progressive course of the disorder. It is worthwhile removing individual large metastases and sometimes even a hemi-hepatectomy is indicated. The operation may allow the patient to be symptom-free for years. However, carcinoid patients are especially at risk during surgery and the symptoms can also be relieved by drugs. The indications for a palliative operation must therefore be very carefully considered.

The carcinoid is not very radio-sensitive, and radiation is therefore not helpful. Cystostatics, such as cyclophosphamide, can result in subjective improvement, objective reduction in the size of the metastases, and regression in the 5-hydroxy indole-acetic acid excretion. A local perfusion of the liver with 5-fluorouracil may occasionally be considered.

The flush is best influenced by a daily dose of 20–40 mg prednisone, which inhibits the activation of kallikrein and thus the formation of bradykinin. The alphareceptor blockers dibenzyline and phentolamine (Regitin) also act symptomatically. Decarboxylase blockers such as alpha-methyl-dopa have no effect. Trasylol, the kallikrein inhibitor, suppresses the flush only during slow infusion. Reserpine cannot be used since it does not deplete the enteral store of serotonin. On the other hand, chlorpromazine can greatly improve the flush, diarrhea, abdominal pain and bronchial spasms, or even cause them to disappear. In addition to being a sedative, chlorpromazine is also an antagonist to bradykinin. Only the diarrhea responds to the serotonin antagonist, methysergide. The use of parachlorophenylalanine is of theoretical interest. It competitively inhibits the hydroxylation of tryptophane to 5-hydroxytryptophane. The administration of several grams daily improves the diarrhea but depletion of the serotonin storage in the central nervous system results in side effects such as depression and hallucinations (ENGELMANN, 1967).

ϑ) Atypical Carcinoid Syndrome

There are carcinoids which are not quite typically situated and in which the histology, clinical symptoms, and biochemistry are also atypical. On the other hand, noncarcinoid tumors may be associated with typical clinical carcinoid symptoms and biochemistry (HEDINGER, 1962; MOERTEL, 1965).

The former are associated with a light red, patchy, often very extensive flush, and besides serotonin, they produce 5-hydroxytryptophane and, especially, histamine which are demonstrable in increased amounts in the blood and urine. The stomach and bronchus are given as the most frequent sites (WALDENSTRÖM, 1963; MELMON, 1965).

The typical carcinoid syndrome may arise in association with carcinomas of the stomach, liver, biliary passages, and pancreas. In most of these cases, an increased formation of serotonin and a raised excretion of 5-hydroxy indole acetic acid can be demonstrated. On the other hand, carcinoids of the ovary can provoke reversible alterations of the heart without any flushes occurring. This suggests that the flushes may not be caused directly by serotonin (CHATTERJEE, 1968).

Finally, there are intermediary stages of the paraneoplastic syndrome in which different tumors, especially the oat-cell bronchus carcinoma, can produce a carcinoid syndrome and other endocrine hyperfunction syndromes such as CUSHING's syndrome at the same time (SIEGENTHALER, 1965). Finally, there are different combined forms of endocrine adenomatoses and Zollinger-Ellison syndrome (WILLIAMS, 1962; SCHMID, 1964).

E. The Prostaglandins

The observation that human seminal fluid contains substances which activate smooth muscle fibers (KURZROK and LIEB, 1930; GÖEDBLATT, 1933; v. EULER, 1934) led to the isolation and the constitutional examination of the prostaglandins (BERGSTRÖM, 1966). Prostaglandins are unsaturated, aromatic, long-chained fatty acids with free hydroxy and keto groups.

Three groups with different actions have been differentiated: prostaglandin E, with a keto group and an alpha-hydroxy on the five-membered ring, prostaglandin F, with two alpha-hydroxyl groups, and prostaglandin A, with a keto group and a double bond on the ring. Within these groups, additional double bonds and hydroxyl groups allow differentiation of E 1–3, F 1–2 and A 1–8, so that around 20 active compounds are now known.

Prostaglandins are found in the seminal fluid of man, monkey, sheep and goat but not in other mammals. They can be extracted from the seminal vesicles of sheep. The physiological significance for the muscular contractions of the uterus or tubes is currently being investigated. They are also found in human menstrual blood as well as in numerous other tissues such as the lungs, kidneys, brain, pancreas, thymus, and iris.

Prostaglandins are composed of essential fatty acids, especially arachidonic acid. They accumulate in the kidneys and liver and are oxidized and quickly metabolized in the lungs and liver (SAMUELSON, 1967). Their physiological importance is presently unknown. Biological actions have been demonstrated on smooth muscle, on the heart, on the vasomotor system, on the kidney, on the lungs, on the gastrointestinal tract, on the fatty tissues, on the blood, and on the central nervous system.

In addition, there is an effect on the female reproductive organs. Hypotheses and facts concerning the physiology are reviewed by HORTON (1969), for current papers see the journal "Prostaglandins", edited by ANDERSON, CALDWELL, and SPEROFF since 1972.

Prostaglandins E and F act very strongly on smooth muscle fibers outside of the vasomotor system, while Prostaglandin A has only a weak effect. As a rule, they produce contractions (diarrhea) but may also induce dilatation, depending on the dose and the state of the muscle (e.g. the uterus). E and A cause the blood pressure to fall; F causes a rise. The peripheral resistance is reduced, independently of the alpha- and beta-receptors and the action of noradrenaline. When PGA is used, no side effects on the gastrointestinal tract arise. The cardiac output increases due to tachycardia.

It is possible that prostaglandin A, which is particularly abundant in the outer layers of the renal medulla ("medullin") represents the renal hypotensive factor. When this factor is lost, renoprival high pressure develops. It inhibits the Goldblatt mechanism in the presence of an intact contralateral kidney. As a renal hormone, PGA may have a generalized vasodilatory action, or it may act locally within the kidney to increase the blood supply to the cortex and cause the loss of sodium in the urine (LEE, 1967).

The relations between prostaglandins and the natriuretic "third factor" (see Chap. VII, p. 301) are controversial. The prostaglandins seem to increase plasma renin activity (CARLSON, 1967; WERNING, 1970) while they also have an antagonistic effect towards angiotensin II (WERNING, 1970).

PGE has an anti-lipolytic action on spontaneous or hormone-induced lipolysis in the fatty tissues of rats *in vitro*. It is possible that this action is due to the cyclase. In the intact animal PGE (but not PGF) in high doses reduces the free fatty acids induced by noradrenaline. Low doses can probably increase them through a sympathetic activation. Finally the prostaglandins in group E act on the central nervous system. They are liberated spontaneously, but especially under electrical stimulation and under the influence of spasmogenic substances. They appear to modify the action of carrier substances without being such substances themselves. E_1 and E_2 have recently been found to be stimulators of bone resorption (RAISZ, 1973).

It is possible that a hyperfunctional syndrome of the prostaglandins exists in medullary carcinoma of the thyroid gland, as well as calcitonin overproduction (see p. 232).

This type of thyroid carcinoma, which is associated with multiple familial phaeochromocytomas and fibromas (see p. 232), is often associated with persistent diarrhea. In 4 of 7 such tumors, prostaglandins were shown to be the active substance (WILLIAMS, 1968).

On the other hand, many tumors of the bronchi and other organs produce prostaglandins which can provoke diarrhea besides other ectopic hormones (SANDLER, 1968). Further, an increased content of prostaglandins has been found in the diseased kidney in renovascular hypertension (STRONG, 1966).

New pharmacological properties with possible therapeutic applications are being discovered all the time.

Prostaglandins are used as hypotensive agents, in the prophylaxis of thrombosis, and in the treatment of peptic ulcer and arthritis. They are currently being tested in the induction of labor and abortion; they are harmless, though not completely free of side effects (diarrhea and vomiting). Particular interest is devoted to their possible application as a contraceptive. Abortion can be induced in the human in early as well as late pregnancy by the infusion of high doses of PGE 1, PGE 2, or PGF2α (EMBREY, 1970; KARIM, 1970). Topical application avoids the side effects. Low doses can be used to induce parturition at term.

PGE 2 stimulates progesterone synthesis *in vitro* (SPEROFF, 1970), whereas PGF 2α *in vivo* has a luteolytic action (MCCRACKEN, 1970; KIRTON, 1970). Probably the reduced blood flow through the ovary causes resorption of the corpus luteum and a fall in the level of progesterone in the blood so that implantation of the ovum in the endometrium does not take place.

All these uses of the prostaglandins are still at the testing stage.

The main physiological or pharmacological effects currently known can be summed up by stating that the prostaglandins influence 1. the smooth muscles of the female reproductive tract, 2. parts of the endocrine system, 3. the adipose tissue, 4. the central nervous system, 5. the peripheral nervous system, 6. the cardiovascular system, 7. the urinary system, 8. the blood platelets, 9. the motility and secretions of the gastrointestinal tract, and 10. the smooth muscles of the respiratory tract.

References

Review Articles

CROXATTO, H., BARNAFI, L.: Hormone and hormonelike activity of active polypeptides. Recent Progr. Hormone Res. **16**, 263 (1960).

DEMLING, L. (ed.): Gastrointestinal hormones. Stuttgart: Thieme 1972.

DIRSCHERL, W.: Hormonbegriff. In: Fermente, Hormone, Vitamine, 3rd ed. Vol. II, Hormone, hrsg. von R. AMMON und W. DIRSCHERL, Eds. Stuttgart: Thieme 1960.

ERSPAMER, V.: Pharmacologically active substances of mammalian origin. Ann. Rev. Pharmacol. **1**, 175 (1961).

GADDUM, J. H.: Polypeptides which stimulate plain muscle. Edinburgh: Livingstone 1955.

GROSSMANN, M. J.: Spectrum of biological actions on gastrointestinal hormones. Nobel symposium XVI. Frontiers in gastrointestinal hormone research, 1970.

HOLTZ, P.: Gewebshormone. In: Fermente, Hormone, Vitamine, 3rd ed. Vol. II, Hormone, hrsg. von R. AMMON und W. DIRSCHERL, Eds. Stuttgart: Thieme 1960.

— Gewebs- und Neurohormone. In: 8. Symp. Dtsch. Ges. Endocr. Berlin-Göttingen-Heidelberg: Springer 1962.

JORPES, J. E., MUTT, V. (eds.): Secretin, Cholecystokinin-Pancreozymin and Gastrin. Handbook of experimental pharmacology. Vol. 34. Berlin-Heidelberg-New York: Springer 1973.

KOLLER, G.: Vergleichende Physiologie der Hormonwirkung. In: Hormone und ihre Wirkungsweise. Berlin-Göttingen-Heidelberg: Springer 1955.

PEARSE, A. G. E., POLAK, J. M.: Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. Gut **12**, 783 (1971).

Gastrin

BEDI, B. S., GILLESPIE, G., GILLESPIE, I. E.: Effects of a specific gastrin antagonist on gastric acid secretion in pouch dogs. Lancet **1967 I**, 1240.

BLACK, J. W., DUNCAN, W. A. M., DURANT, C. J., GANELLIN, C. R., PARSON, E. M.: Definition and antagonism of histamine H_2-receptors. Nature (Lond.) **236**, 385 (1972).

BYRNES, D. J., YOUNG, J. D., CHISHOLM, D. J., LAZARUS, L.: Serum gastrin in patients with peptic ulceration. Brit. med. J. **1970 II**, 626.

CODE, CH. F.: Histamine and gastric secretion: a later look, 1955—1965. Fed. Proc. **24**, 1311 (1965).

COHEN, S.: Hypogastrinemia and sphincter incompetence. New Engl. J. Med. **289**, 215 (1973).

GANGULI, P. C., CULLEN, D. R., IRVINE, W. J.: Radioimmunoassay of plasma-gastrin in pernicious anemia, achlorhydria without pernicious anemia, hypochlorhydria, and in controls. Lancet **1971 I**, 155.

GREGORY, R. A.: Gastrin—The natural history of a peptide hormone. Harvey lectures, series 64, 121. New York and London: Academic Press 1970.

— TRACY, H. J.: The constitution and properties of two gastrins extracted from hog antral mucosa. Gut **5**, 103 (1964).

— — Isolation of two "big gastrins" from Zollinger-Ellison tumors tissue. Lancet **1972 II**, 797.

GROSSMANN, M. I.: Gastrin. Berkeley and Los Angeles. University of California Press 1966.

— Gastrin and its activities. Nature (Lond.) **228**, 1147 (1970).

HAEMMERLI, U. P.: Pathophysiologie des Antrums. Schweiz. med. Wschr. **98**, 242 (1968).

HAKANSON, R., LIEDBERG, G.: Evidence against histamine as final chemostimulator of gastric acid secretion. Amer. J. Physiol. **221**, 641 (1971).

HALTER, F., FUNK, H. U.: Der Pentagastrintest als klinischer Routinetest zur Bestimmung der Magensäuresekretion. Schweiz. med. Wschr. **98**, 1149 (1968).

HANSKY, J., ROYLE, J. P., SOVENY, C., KORMAN, M. G.: Relationship of immunoreactivity to biological activity of gastrin. Gastroenterology **64**, 739 (1973).

HUGHES, W. S., BROOKS, F. P., COHN, H. O.: Serum gastrin levels in primary hypogammaglobulinemia and pernicious anemia. Studies in adults. J. clin. Invest. **77**, 746 (1972).

KORMAN, M. G., LAVER, M. C., HANSKY, J.: Hypergastrinemia in chronic renal failure. Brit. med. J. **1972 I**, 209.

MCGUIGAN, J. E., TRUDEAU, W. L.: Immunochemical measurement of elevated levels of gastrin in the serum of patients with pancreatic tumors of the Zollinger-Ellison variety. New Engl. J. Med. **278**, 1308 (1968).

— — Serumgastrin concentration in pernicious anemia. New Engl. J. Med. **282**, 358 (1970).

Multicentre pilot study: Pentagastrin as a stimulant of maximal gastric acid response in man. Lancet **1967 I**, 291.

OTTENJAHN, R.: Antigastrine und Gastrin-Antikörper. Dtsch. med. Wschr. **93**, 1773 (1968).

SEELIG, H. P.: Gastrin. Inaktivierung und Abbau. Stuttgart: Thieme 1972.

SILEN, W.: Advances in gastric physiology. New Engl. J. Med. **277**, 864 (1967).

TRACY, H. J., GREGORY, R. A.: Physiological properties of a series of synthetic peptides structurally related to gastrin I. Nature (Lond.) **204**, 935 (1964).

TRUDEAU, W. L., MCGUIGAN, J. E.: Effects of calcium on serum gastrin levels in the Zollinger-Ellison syndrome. New Engl. J. Med. **281**, 802 (1969).

— — Relations between serum gastrin levels and rates of gastric hydrochloric acid secretion. New Engl. J. Med. **284**, 408 (1971).

WALSH, J. H., DEBAS, H. T., GROSSMAN, M. I.: Pure natural human big gastrin: biological activity and half life in dog. Gastroenterology **64**, 873 (1973).

WORMSLEY, K. G.: Pentagastrin snuff. Lancet **1968 I**, 57.

YALOW, R. S., BERSON, S. A.: Further studies on the nature of immunoreactive gastrin in human plasma. Gastroenterology **60**, 203 (1971).

— Wu, N.: Additional studies on the nature of big big gastrin. Gastroenterology **65**, 19 (1973).

Zollinger-Ellison Syndrome

Amberg, J. R., Ellison, E. H., Wilson, S. D., Zboralske, F. F.: Roentgenographic observations in the Zollinger-Ellison syndrome. J. Amer. med. Ass. **190**, 185 (1964).
Aoyagi, T., Summerskill, W. H. I.: Gastric secretion with ulcerogenic islet cell tumor: importance of basal acid output. Arch. intern. Med. **117**, 667 (1966).
Bretholz, A., Steiner, H.: Les insulomes. Intérêt d'un diagnostic histologique précis. Virchows Arch. Abt. A. Path. Anat. **359**, 49 (1973).
Cavallero, C., Solcia, E., Sampietro, R.: Cytology of islet tumours and hyperplasia associated with the Zollinger-Ellison syndrome. Gut **8**, 172 (1967).
Christoforidis, A. J., Nelson, S. W.: Radiological manifestations of ulcerogenic tumors of the pancreas. J. Amer. med. Ass. **198**, 511 (1966).
Clemett, A. R., Park, W. M.: Arteriographic demonstration of pancreatic tumor in the Zollinger-Ellison syndrome. Radiology **88**, 32 (1967).
Creutzfeldt, W., Arnold, R., Creutzfeldt, C., Denticke, U., Frerichs, H., Track, N. S.: Biochemical and morphological investigations of 30 human insulinomas. Diabetologia **9**, 217 (1973).
Demling, L., Ottenjahn, R.: Non-insulin producing tumors of the pancreas. Modern aspects on Zollinger-Ellison syndrome and gastrin. Stuttgart: Thieme 1969.
Editorial: Zollinger-Ellison syndrome revisted. Brit. med. J. **1973 I**, 2.
Enderlin, F.: Das Zollinger-Ellison-Syndrom. Ergebn. Chir. Orthop. **44**, 112 (1962).
Gregory, R. A., French, J. M., Sircus, W.: Extraction of a gastrin-like substance from a pancreatic tumor in a case of Zollinger-Ellison syndrome. Lancet **1960 I**, 1045.
Hallenbeck, G. A.: The Zollinger-Ellison syndrome. Gastroenterology **54**, 426 (1968).
Isenberg, J. I., Walsh, J. H., Grossman, M. I.: Zollinger-Ellison syndrome. Gastroenterology **65**, 140 (1973).
— — Passaro, E., Jr., Moore, E. W., Grossman, M. I.: Unusual effect of secretin on serum gastrin, serum calcium and gastric acid secretion in a patient with suspected Zollinger-Ellison syndrome. Gastroenterology **62**, 626 (1972).
Keel, H. J., Roth, J. L. A.: Nicht Insulin produzierende Inselzelltumoren. Schweiz. med. Wschr. **42**, 1399 (1967).
Marks, I. N., Bank, S., Louw, J. H.: Islet cell tumor of the pancreas with reversible watery diarrhea and achlorhydria. Gastroenterology **52**, 695 (1967).
McGuigan, J. E., Trudeau, W. L.: Immunochemical measurement of elevated levels of gastrin in the serum of patients with pancreatic tumors of the Zollinger-Ellison variety. New Engl. J. Med. **278**, 1308 (1968).
Mieher, W. C., Jr., Hartsock, R. J., Geokas, M. C., Ballard, H. S., Frame, B.: Peptic ulcer as a manifestation of familial polyendocrine disease. Report of 2 cases occurring in sisters, with a preliminary report of 8 other family members. J. Amer. med. Ass. **179**, 854 (1962).
Osborne, M. P., Brown, M. E., Le Compte, P. M.: Ulcerogenic non-beta-cell pancreatic islet carcinoma. Amer. J. Surg. **100**, 48 (1960).
Passaro, E., Jr., Basso, N., Sanchez, R. E., Gordon, H. E.: Newer studies in the Zollinger-Ellison syndrome. Amer. J. Surg. **120**, 138 (1970).
— — Walsh, J. H.: Calcium challenge in the Zollinger-Ellison syndrome. Surgery **72**, 60 (1972).
Perrier, C. V.: The Zollinger-Ellison syndrome, its place in the pathophysiology of gastric acid secretion and of its hormonal regulation. Ergebn. inn. Med. Kinderheilk. **23**, 89 (1965).
Polak, J. M., Stagg, B., Pearse, A. G. E.: Two types of Zollinger-Ellison syndrome: immunofluorescent, cytochemical and ultrastructural studies of the antral and pancreatic gastrin cells in different clinical states. Gut **13**, 501 (1972).
Royston, C. M. S., Brew, D. St. J., Garnham, J. R., Stagg, B. H., Polak, J.: The Zollinger-Ellison syndrome due to an infiltrating tumour of the stomach. Gut **13**, 638 (1972).
Winship, D. H., Ellison, E. H.: Variability of gastric secretion in patients with and without the Zollinger-Ellison syndrome. Lancet **1967 I**, 1128.
Zollinger, R. M., Craig, Th. V.: Endocrine tumors and peptic ulcer. Amer. J. Med. **29**, 761 (1960).
— Ellison, E. H.: Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. Ann. Surg. **142**, 709 (1960).
— Grant, G. N.: Ulcerogenic tumor of the pancreas. J. Amer. med. Ass. **190**, 181 (1964).

WDHA Syndrom

Bloom, S. R., Polak, J. M., Pearse, A. G. E.: Vasoactive intestinal peptide and watery-diarrhoea syndrome. Lancet **1973 II**, 16.
Brown, Ch. H., Crile, G.: Pancreatic adenoma with intractable diarrhea, hypokalemia and hypercalcemia. J. Amer. med. Ass. **190**, 30 (1964).
Elias, E., Bloom, S. R., Welbourn, R. B., Kozio, M., Polak, J. M., Pearse, A. G. E., Booth, C. C., Brown, J. C.: Pancreatic cholera due to production of gastric inhibitory polypeptide. Lancet **1972 II**, 791.
Knappe, G., Flemming, F., Stobbe, H., Wendt, F.: Pankreasinselzelladenom mit der Trias Diarrhoe, Hypokaliämie und Hyperglykämie. Dtsch. med. Wschr. **27**, 1224 (1966).
Matsumoto, K. K., Peter, J. B., Schultze, R. G., Hakim, A. A., Franck, P. T.: Watery diarrhea and hypokalemia associated with pancreatic islet cell adenoma. Gastroenterology **50**, 231 (1966).
Verner, J. V., Morrison, A. B.: Islet cell tumor and a syndrome of refractory watery diarrhea and hypokalemia. Amer. J. Med. **25**, 456 (1958).
Zollinger, R. M., Thompkins, R. K., Amerson, J. R., Endahl, G. L., Kraft, A. R., Moore, F. T.: Identification of the diarrheogenic hormone associated with non-beta cell tumors of the pancreas. Ann. Surg. **168**, 502 (1968).

Secretin, Cholecystokinin, Pancreomyzin, Enterogastron, Caerulein, VIP, GIP, Motilin

Anastasi, A., Erspamer, V., Endean, R.: Isolation and structure of caerulein, an active decapeptide from the skin of Hyla caerulea. Experientia (Basel) **23**, 699 (1967).
Bernardi, L., Bosisio, G., Castiglione, R., De Goffredo, O.: Synthesis of caerulein Experientia (Basel) **23**, 700 (1967).
Brooks, A. M., Agosti, A., Bertaccini, G., Grossman, M. I.: Inhibition of gastric acid secretion in man by peptide analogues of cholecystokinin. New Engl. J. Med. **282**, 535 (1970).
Brown, J. C., Cook, M. A., Dryburgh, J. R.: Motilin, a gastric motor activity-stimulating polypeptide: final purification, amino acid composition, and C-terminal residues. Gastroenterology **62**, 401 (1972).
— Dryburgh, J. R.: A gastric inhibitory polypeptide II: The complete amino acid sequence. Canad. J. Biochem. **49**, 867 (1971).
Castell, D. O., Harris, L. D.: Hormonal control of gastroesophageal sphincter strength. New Engl. J. Med. **282**, 866 (1970).

DREILING, D.A.: The measurement of pancreatic secretory function. In: Exocrine pancreas. Ciba foundation, p. 225. London: Churchill 1962.
ERSPAMER, V.: Progress report: Caerulein. Gut **11**, 79 (1970).
GREGORY, R.A.: Secretory mechanism of the gastrointestinal tract. London: Arnold 1962.
HARPER, A.A., RAPER, H.S.: Pancreozymin, stimulant of secretion of pancreatic enzymes in extracts of small intestine. J. Physiol. (Lond.) **102**, 115 (1943).
ISENBERG, J.I., BRICKMAN, A.S., MOORE, E.W.: The effect of secretin on serum calcium in man. J. clin. Endocr. **37**, 30 (1973).
IVY, A.C., OLDBERG, E.: Hormone mechanism for gall-bladder contraction and evacuation. Amer. J. Physiol. **86**, 599 (1928).
JOHNSON, L.R., GROSSMANN, M.I.: Intestinal hormones as inhibitors of gastric secretion. Gastroenterology **60**, 120 (1971).
JORPES, E., MUTT, V.: Gastrointestinal hormones. In: The hormones, vol. IV, PINCUS, THIMANN and ASTWOOD, Eds. New York: Academic Press 1964.
JORPES, J.E.: Memorial lecture—isolation and chemistry of secretin and cholecystokinin. Gastroenterology **55**, 157 (1968).
MEYER, J.H., GROSSMAN, M.I.: Release of secretin and cholecystokinin. In: Gastrointestinal hormones, DEMLING, L. (ed.), p. 43. Stuttgart: Thieme 1972.
MORISSET, J.A., WEBSTER, P.D.: In vitro and in vivo effects of Urecholine and cyclic AMP on rat pancreas. Amer. J. Physiol. **220**, 202 (1971).
MUTT, V.: Some recent developments in the field of the intestinal hormones. In: Endocrinology (1971). Proc. 1st Internat. Sympos. 1971. London: Heinemann 1972.
— JORPES, J.E.: Contemporary developments in the biochemistry of the gastrointestinal hormones. Recent Progr. Hormone Res. **23**, 483 (1967).
OTTENJANN, R.: Ein neues Dünndarmhormon. Dtsch. med. Wschr. **96**, 838 (1971).
PEDERSON, R.A., BROWN, J.C.: Inhibition of histamine-pentagastrin- and insulin-stimulated canine gastric secretion by pure "gastric inhibitory polypeptide". Gastroenterology **62**, 393 (1972).
SAID, S.I., MUTT, V.: Polypeptide with broad biological activity: isolation from small intestine. Science **169**, 1217 (1970).
— — Potent peripheral and splanchnic vasodilator peptide from normal gut. Nature **225**, 863 (1970).

Plasmakinins

ERDÖS, E.G.: Hypotensive peptides: Bradykinin, Kallidin and Eledoisin. In: Advances in pharmacology, Vol. 4, GARATTINI and SHORE, Eds. New York and London: Academic Press 1966.
— Bradykinin, Kallidin and Kallikrein. Handbuch der experimentellen Pharmakologie, Vol. XXV. Berlin-Heidelberg-New York: Springer 1970.
— BACK, N., SICUTERI, F., WILDE, A.F. (Eds.): Hypotensive peptides. Berlin-Heidelberg-New York: Springer 1966.
HABERMANN, E.: Fortschritte auf dem Gebiete der Plasmakinine. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. **245**, 230 (1963).
KELLERMEYER, R.W., GRAHAM, R.C., JR.: Kinins—possible physiologic and pathologic roles in man. New Engl. J. Med. **279**, 754, 859 (1968).
LEWIS, G.P.: Active polypeptids derived from plasma-proteins. Phys. Rev. **40**, 647 (1960).
MELMON, K.L., CLINE, M.J.: Kinins. Amer. J. Med. **43**, 153 (1967).
SCHACHTER, H.: Symposium "Vasoactive peptides". Fed. Proc. **27**, 49 (1968).
SCHACHTER, M.: Polypeptides which affect smooth muscles and blood vessels. Oxford: Pergamon Press 1960.
— Kinins—a group of active peptides. Ann. Rev. Pharmacol. **4**, 281 (1964).
— Kallikreins and Kinins. Physiol Rev. **49**, 509 (1969).
STÜRMER, E.: Plasmakinine. Schweiz. med. Wschr. **96**, 1667 (1966).
WERLE, E.: Plasmakinine. Dtsch. med. Wschr. **92**, 1573 (1967).

Renin-Angiotensin II

Reviews

BROWN, J.J., DAVIES, D.L., LEVER, A.F., ROBERTSON, J.I.S.: Renin and angiotensin. A survey of some aspects. Postgrad. med. J. **42**, 153 (1966).
FISHER, J.W. (ed.): Kidney hormones. London and New York: Academic Press 1971.
GROSS, F.: Renin und Hypertensin, physiologische oder pathologische Wirkstoffe? Klin. Wschr. **36**, 693 (1958).
— BRUNNER, H., ZIEGLER, M.: Renin-angiotensin system, aldosterone, and sodium balance. Recent Progr. Hormone Res. **21**, 119 (1965).
PEART, W.S.: The functions for renin and angiotensin. Recent Progr. Hormone Res. **21**, 73 (1965).
— The renin-angiotensin system. Pharmacol. Rev. **17**, 144 (1965).
— Renin and angiotensin. In: Hormones in the blood, C.H. GRAY and A.L. BACHARACH, Eds. 2nd ed., Vol. 2, p. 489. London: Academic Press 1967.
Proceedings of the International Symposium on Angiotensin, Sodium and Hypertension. In: Canad. med. Ass. J. **90**, 153—341 (1964).

Biochemistry

BIRON, P.: Les angiotensinases du plasma humain. Rev. canad. Biol. **26**, 43 (1967).
BROWN, J.J., DAVIES, D.L., LEVER, A.F., ROBERTSON, J.I.S., TREE, M.: The estimation of renin in human plasma. Biochem. J. **93**, 594 (1964).
CARRETERO, O., GROSS, F.: Renin substrate in plasma under various experimental conditions in the rat. Amer. J. Physiol. **213**, 695 (1967).
DEODHAR, S.D., HAAS, E., GOLDBLATT, H.: Production of antirenin to homologous renin and its effect on experimental renal hypertension. J. exp. Med. **119**, 425 (1964).
GOULD, A.B., SKEGGS, L.T., KAHN, J.R.: Measurement of renin and substrate concentrations in human serum. Lab. Invest. **15**, 1802 (1966).
HAAS, E., GOLDBLATT, H., GIPSON, E.C.: Extraction, purification, and acetylation of human renin and the production of antirenin to human renin. Arch. Biochem. **110**, 534 (1965).
LARAGH, J.H., SEALEY, J.E., LEDINGHAM, J.G.G., NEWTON, M.A.: Oral contraceptives. Renin, aldosterone and high blood pressure. J. Amer. med. Ass. **201**, 918 (1967).
NG, K.K.F., VANE, J.R.: Conversion of angiotensin I to angiotensin II. Nature **216**, 762 (1967).
PAGE, I.H., BUMPUS, F.M.: Angiotensin—a renal hormone. Recent Progr. Hormone Res. **18**, 167 (1962).
SKEGGS, L.T., LENTZ, K.E., GOULD, A.B., HOCHSTRASSER, H., KAHN, J.R.: Biochemistry and kinetics of the renin-angiotensin system. Fed. Proc. **26**, 42 (1967).
— — HOCHSTRASSER, H., KAHN, J.R.: The purification and partial characterization of several forms of hog renin substrate. J. exp. Med. **118**, 73 (1963).

Physiology

BROWN, J.J., LEVER, A.F., ROBERTSON, J.I.S.: Renin and angiotensin in health and disease. Schweiz. med. Wschr. **97**, 1635, 1679 (1967).

DOUGLAS, W.W.: Angiotensin. In: The pharmacological basis of therapeutics, L.S. GOODMAN and A. GILMAN, Eds. 3rd ed., p. 653. New York: Macmillan 1965.

GORDON, R.D., WOLFE, L.K., ISLAND, D.P., LIDDLE, G.W.: A diurnal rhythm in plasma renin activity in man. J. clin. Invest. **45**, 1587 (1966).

GROSS, F.: The regulation of aldosterone secretion by the renin-angiotensin system under various conditions. Acta endocr. (Kbh.), Suppl. **124**, 41 (1967).

KLAUS, D.: Regulation der Reninsekretion. Dtsch. med. Wschr. **92**, 2126 (1967).

— HEIZMANN, A.: Zur Regulation der Reninsekretion durch Angiotensin. Klin. Wschr. **45**, 657 (1967).

KOCH-WESER, J.: Myocardial actions of angiotensin. Circulat. Res. **14**, 337 (1964).

LICHTLEN, P., SCHAUB, F., BÜHLMANN, A.: Experimentelle Untersuchungen und therapeutische Erfahrungen mit Angiotensin. Schweiz. med. Wschr. **92**, 639 (1962).

VANDER, A.J.: Control of renin release. Physiol. Revs. **47**, 359 (1967).

VEYRAT, R., CHAMPLAIN, J. DE, BOUCHER, R., KOIW, E., GENEST, J.: Le rôle du système rénine-angiotensine dans la régulation du métabolisme du sodium chez l'homme. Schweiz. med. Wschr. **94**, 914 (1964).

WOLFF, H.P.: Aldosteron und Aldosteronismus. Neue Ergebnisse und Erfahrungen. Klin. Wschr. **42**, 711 (1964).

Clinical Syndromes

BROWN, J.J., CHINN, R.H., FRASER, R., LEVER, A.F., MORTON, J.J., ROBERTSON, J.I.S., TREE, M., WAITE, M.A., PARK, D.M.: Recurrent hyperkalaemia due to selective aldosterone deficiency: Correction by angiotensin infusion. Brit. med. J. **1973 I**, 650.

CONN, J.W., COHEN, E.L., LUCAS, CH.P., MCDONALD, W.J., MAYOR, G.H., BLOUGH, W.M., EVELAND, W.C., BOOKSTEIN, J.J., LAPIDES, J.: Primary reninism. Arch. intern. Med. **130**, 682 (1972).

Leading article: Primary excess and deficiency of renin. Brit. med. J. **1973 I**, 627.

Methods of Determination

See Chap. VII, p.

Substance P

BAILE, C.A., MEINARDI, H.: Action of substance P on the central nervous system of a goat. Brit. J. Pharmacol. **30**, 302 (1967).

CHANG, M.M., LEEMAN, S.E., NIALL, H.D.: Amino-acid sequence of substance P. Nature New Biol. **232**, 86 (1971).

EULER, U.S. VON, GADDUM, J.H.: An unidentified depressor substance in certain tissue extracts. J. Physiol. (Lond.) **72**, 74 (1931).

HAEFELY, W., HÜRLIMANN, A.: Substance P, a highly active naturally occurring polypeptide. Experientia (Basel) **18**, 297 (1962).

LEMBECK, F., ZETLER, G.: Substance P, a polypeptide of possible physiological significance, especially within the nervous system. Int. Rev. Neurobiol. **4**, 159 (1962).

MEINARDI, H., CRAIG, L.C.: Studies of substance P. In: ERDÖS, E.G., BACK, N., SICUTERI, F., WIEDE, A.F. (Eds.), Hypotensive peptides, p. 594. Berlin-Heidelberg-New York: Springer 1966.

PERNOW, B.: Studies on substance P. Purification, occurrence and biological actions. Acta physiol. scand. **29**, Suppl. 105, 1 (1953).

STUDER, R.O., TRZECIAK, H., LERGIER, W.: Isolierung und Aminosäuresequenz von Substanz P aus Pferdedarm. Helv. chim. Acta **56**, 860 (1973).

TREGEAR, G.W., NIALL, H.D., POTTS, J.T., JR., LEEMAN, S.E., CHANG, M.M.: Synthesis of substance P. Nature New Biol. **232**, 87 (1971).

VOGLER, K., HAEFELY, W., HÜRLIMANN, A., STUDER, R.O., LERGIER, W., STRÄSSLE, R., BERNEIS, K.H.: A new purification procedure and biological properties of substance P. Ann. N.Y. Acad. Sci. **104**, 378 (1963).

ZETLER, G.: Substanz P. Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. **245**, 263 (1963).

VEM and VDM

SHORR, E., MAZUR, A., BAEZ, S.: Chemical and biological properties of the hepatorenal factors VEM and VDM (Ferritin). Recent Progr. Hormone Res. **11**, 453 (1955).

Parotin

COHEN, S.: Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. Proc. nat. Acad. Sci. (Wash.) **46**, 302 (1960).

ITO, Y.: Parotin: a salivary gland hormone. Ann. N.Y. Acad. Sci. **85**, 228 (1960).

LEVI-MONTALCINI, R., ANGELETTI, P.U.: Nerve growth factor. Physiol. Rev. **48**, 534 (1968).

Erythropoietin

ADAMSON, J.W., ALEXANIAN, R., MARTINEZ, C., FINCH, C.A.: Erythropoietin excretion in normal man. Blood **28**, 354 (1966).

— ESCHBACH, J., FINCH, C.A.: The kidney and erythropoiesis. Amer. J. Med. **44**, 725 (1968).

— FINCH, C.A.: Erythropoiétine et Erythropoièse. (Editorial.) Nouv. Rev. franç. Hémat. **7**, 157 (1967).

ERSLEV, A.J.: The search for Erythropoietin. New Engl. J. Med. **284**, 849 (1971).

FISHER, I.W.: Kidney hormones. London-New York: Academic Press 1971.

GIGER, K.: Sekundäre Polyglobulic bei Nierentumoren. Schweiz. med. Wschr. **97**, 1067 (1967).

GURNEY, C.W.: Erythropoietin. Adv. in metabolic disorders **3**, 279 (1968).

KUBANEK, B.: Erythropoietin: The hematologist's hormone. Horm. Metab. Res. **1**, 151 (1969).

PENINGTON, D.G.: The relation of erythropoietin to polycythemia. Proc. roy. Soc. Med. **59**, 1091 (1967).

REMMELE, W.: Die humorale Steuerung der Erythropoiese. Berlin-Göttingen-Heidelberg: Springer 1963.

ROSSE, W.F., WALDMANN, T.A.: The metabolism of erythropoietin in patients with anemia due to deficient erythropoiesis. J. clin. Invest. **43**, 1348 (1948).

— — COHEN, P.: Renal cysts, erythropoietin and polycythemia. Amer. J. Med. **34**, 76 (1963).

SCHOOLEY, S.C., GARCIA, S.F.: Immunologic studies on the mechanism of action of erythropoietin. Proc. Soc. exp. Biol. (N.Y.) **110**, 636 (1962).

SCHULZ, E.: Die klinische Bedeutung des Erythropoetins. Internist (Berl.) **12**, 210 (1971).

STOHLMANN, F.: Erythropoiesis. New Engl. J. Med. **267**, 342, 392 (1962).

— Pathogenesis of erythrocytosis. Seminars in Hematology **3**, 181 (1966).

WHITE, E.F., GUERNY, C.W., GOLDWASSER, E., JACOBSON, L.O.: Studies on erythropoietin. Recent Progr. Hormone Res. **16**, 219 (1960).

Serotonin

ERSPAMER, V.: 5-Hydroxytryptamine and related indolealkylamines. Handbuch experimentelle Pharmakologie, Vol. XIX, EICHLER and FARAH, Eds. Berlin-Heidelberg-New York: Springer 1966.

FEYRTER, F.: Über die peripheren endokrinen (parakrinen) Drüsen des Menschen. Wien: Wilhelm Maudrich 1953.

FRANCHIMONT, P., DELWAIDE, P.J.: Action vasculaire de la serotonine chez l'homme. Rev. franc. Étud. clin. biol. **11**, 876 (1966).

KOELLA, W. P.: Serotonin oder Somnotonin? Schweiz. med. Wschr. **100**, 357 (1970).

Leading article: Serotonin, mental state and behaviour. New Engl. J. Med. **277**, 1146 (1967).

LEMBECK, F.: Physiologie und Pharmakologie des Serotonins. In: Gewebs- und Neurohormone — Physiologie des Melanophorenhormons. 8. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1962.

LEWIS, G. P.: 5-Hydroxytryptamine (Serotonin, Enteramin, 5HT). In: The hormones, Vol. IV, PINCUS, THIEMANN, ASHWOOD, Eds. New York: Academic Press 1964.

SOKOLOFF, B.: Carcinoid and serotonin. Recent results in cancer research, vol. 15. Berlin-Heidelberg-New York: Springer 1968.

UDENFRIEND, W., SHORE, P.A., BOGDANSKI, C.F., BRODIE, B.B.: Biochemical, physiological and pharmacological aspects of serotonin. Recent Progr. Hormone Res. **13**, 1 (1957).

Carcinoid Syndrome

ADAMSON, A.R., GRAHAME-SMITH, D.G., BOGOMOLETZ, V., MAW, D.S.J., ROTHNIE, N.G.: Malignant argentaffinoma with a carcinoid syndrome and hypoglycaemia. Brit. med. J. **1971 3**, 93.

BAYER, O., KOLMAR, D., DONKAS, CHR.: Zur Genese der Herzklappenveränderungen beim Karzinoidsyndrom. Ein Fall von Ovarialkarzinom mit Tricuspidal- und Pulmonalvitium ohne Serotoningradient an der Lungenstrombahn. Dtsch. med. Wschr. **91**, 1217 (1966).

BIÖRCK, G., AXEN, O., THORSON, A.: Unusual cyanosis in a boy with congenital pulmonary stenosis and tricuspid insufficiency. Fatal outcome after angiocardiography. Amer. Heart. J. **44**, 143 (1952).

CASSIDY, M.A.: Abdominal carcinomatosis with probable adrenal involvement. Proc. roy. Soc. Med. **24**, 139 (1930/31).

CHATTERJEE, K., HEATHER, J.C.: Carcinoid heart disease from primary ovarian carcinoid tumors. Amer. J. med. **45**, 643 (1968).

Editorial: Humoral basis of carcinoid flush. Lancet **1966II**, 1013.

ENGELMAN, K., LOVENBERG, W., SJOERDSMA, A.: Inhibition of serotonin synthesis by para-chlorphenylalanine in patients with the carcinoid syndrome. New Engl. J. Med. **277**, 1103 (1967).

GÖSCHKE, H.: Pathophysiologie und medikamentöse Beeinflussung des Karzinoidsyndroms. Schweiz. med. Wschr. **97**, 1548 (1967).

HARRISON, M.T., MONTGOMERY, D.A.D., RAMSEY, A.S., ROBERTSON, J.H., WELBOURN, R.B.: Cushing's syndrome with carcinoma of the bronchus and with features suggesting carcinoid tumor. Lancet **1957I**, 23.

HEDINGER, CHR.: Karzinoidsyndrom und Serotonin. Helv. med. Acta **25**, 351 (1958).

— Die Pathologie des Karzinoidsyndroms und seiner Grenzgebiete. In: Gewebe- und Neurohormone — Physiologie des Melanophorenhormones. 8. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1962.

— GLOOR, R.: Metastasierende Dünndarmkarzinoide, Tricuspidalklappenveränderungen und Pulmonalstenose — ein neues Syndrom. Schweiz. med. Wschr. **84**, 942 (1954).

ISLER, P., HEDINGER, CHR.: Metastasierendes Dünndarmkarzinoid mit schweren, vorwiegend das rechte Herz betreffenden Klappenfehlern und Pulmonalstenose — ein eigenartiger Symptomenkomplex? Schweiz. med. Wschr. **83**, 4 (1953).

KÄHLER, H.J.: Das Karzinoid. Berlin-Heidelberg-New York: Springer 1967.

KIND, H., SCHNEIDER, H.: Serotonin (5-Oxytryptamin) und psychopathologische Erscheinungen. Psychiatrische Befunde beim Dünndarm-Karzinoid. Dtsch. med. Wschr. **82**, 1731 (1957).

KOWLESSAR, O.D., LAW, D.H., SLEISENGER, M.H.: Malabsorption syndrome associated with metastatic carcinoid tumor. Amer. J. Med. **27**, 673 (1959).

LANGEMANN, H., GOERRE, J.: Über das Verhalten der Hydroxyindolessigsäure-Ausscheidung im Urin nach Reserpin beim Menschen. Schweiz. med. Wschr. **87**, 607 (1957).

LEMBECK, F.: Biochemie und Pharmakologie der Carcinoide. Verh. dtsch. Ges. inn. Med. **68**, 194 (1962).

MASON, D.T., MELMON, K.L.: New understanding of the mechanism of the carcinoid flush. Ann. intern. Med. **65**, 1334 (1966).

MELMON, K.L., SJOERDSMA, A., MASON, D.T.: Distinctive clinical and therapeutic aspects of the syndrome associated with bronchial carcinoid tumors. Amer. J. Med. **39**, 568 (1965).

MENGEL, C.E.: Therapy of the malignant carcinoid syndrome. Ann. intern. Med. **62**, 587 (1965).

MENGEL, CH. E., LOTITO, C.A.: A new anti-serotonin in the carcinoid syndrome. Arch. intern. Med. **121**, 507 (1968).

MOERTEL, CH. G., BEAHRS, O.H., WOOLNER, L.B., TYCE, G.M.: Malignant carcinoid syndrome associated with noncarcinoid tumors. New Engl. J. Med. **273**, 244 (1965).

OATES, J.A., PETTINGER, W.A., DOCTOR, R.B.: Evidence for the release of bradykinin in carciniod syndrome. J. clin. Invest. **45**, 173 (1966).

PEART, W.S.: Carcinoid tumors. Acta med. scand., Suppl. **445**, 371 (1966).

POLLARD, A., GRAINGER, R.G., FLEMING, O., MEACHIM, G.: An unusual case of metastasising bronchial adenoma associated with the carcinoid syndrome. Lancet **1962II**, 1084.

ROBERTS, W.C., SJOERDSMA, A.: The cardiac disease associated with the carcinoid syndrome (carcinoid heart disease). Amer. J. Med. **36**, 5 (1964).

SCHMID, M., WENZL, H., UEHLINGER, E.: B-Inselzelladenom des Pankreas mit Hypoglykämie, kombiniert mit multiplen Karzinoidtumoren des Ileum. Schweiz. med. Wschr. **93**, 444 (1963).

SCHNEIDER, H.: Die Psychopathologie des 5-Hydroxytryptamin-(Serotonin)-Stoffwechsels, speziell beim Dünndarmkarzinoid. Schweiz. Arch. Neurol. Psychiat. **81**, 344 (1958).

SHAMES, J.M., DHURANDNAR, N.R., BLACKARD, W.G.: Insulin-secreting bronchial carcinoid tumor with widespread metastases. Amer. J. Med. **44**, 632 (1968).

SIEGENTHALER, D., MERKI, W., FUNK, H.U., HEDINGER, CHR., PLETSCHER, A.: Atypisches Karzinoidsyndrom bei kleinzelligem Bronchuskarzinom. Schweiz. med. Wschr. **95**, 869 (1965).

SJOERDSMA, A., MELMON, K.L.: The carcinoid spectrum. Gastroenterology **47**, 104 (1964).

— WEISSBACH, H., UDENFRIEND, S.: A clinical physiological and biochemical study of patients with malignant carcinoid (Argentaffinoma). Amer. J. Med. **20**, 520 (1956).

SOKOLOFF, B.: Carcinoid and serotonin. Recent results in cancer research, Vol. 15. Berlin-Heidelberg-New York: Springer 1968.

THORSON, A. H.: Studies on carcinoid disease. Acta med. scand., Suppl. **334** (1958).
UDENFRIEND, S., TITUS, S., WEISSBACH, H.: The identification of 5-hydroxy 3-indolacetic acid in normal urine, a method for its assay. J. biol. Chem. **216**, 499 (1955).
— WEISSBACH, H., BRODIE, B. B.: Assay of serotonin and related metabolites, enzymes and drugs. Meth. biochem. Anal. **6**, 95 (1958).
— — CLARK, C. T.: The estimation of 5-hydroxytryptamine (serotonin) in biological tissues. J. biol. Chem. **215**, 337 (1955).
WALDENSTRÖM, J.: Klinik des Carcinoidsyndroms. Verh. dtsch. Ges. inn. Med. **68**, 211 (1962).
WILLIAMS, E. D., CELESTIN, L. R.: The association of bronchial carcinoid and pluriglandular adenomatosis. Thorax **17**, 129 (1962).
WONG, V. G., MELMON, K. L.: Ophthalmic manifestations of the carcinoid flush. New Engl. J. Med. **277**, 406 (1967).
ZEITLIN, I. J., SMITH, A. N.: 5-Hydroxyindoles and kinins in the carcinoid and dumping syndromes. Lancet **1966II**, 986.
ZIEGLER, W. H., LANGEMANN, H., MÜLLER, P. B.: Vergleichende Untersuchungen am Tumorgewebe von Phäochromocytom und Carcinoid. Schweiz. med. Wschr. **97**, 1731 (1967).

Prostaglandins

BERGSTRÖM, S.: The prostaglandins. Recent Prog. Hormone Res. **22**, 153 (1966).
— Prostaglandins: members of new hormonal system. Science **157**, 382 (1967).
— CARLSON, L. A., WEEKS, J. R.: The prostaglandins, a family of biologically active lipids. Pharmacol. Rev. **20**, 1 (1968).
— (ed.) Advances in the Biosciences 9. International conference on prostaglandins. New York, Oxford, Braunschweig: Pergamon/Vieweg 1973.
BÖHLE, E., DÖBERT, E., AMMON, J., DITSCHUNEIT, H.: Über Stoffwechselwirkungen von Prostaglandinen. I. Der Einfluß von Prostaglandin E_1 auf den Glucose- und Fettstoffwechsel des epididymalen Fettgewebes der Ratte. Diabetologia **2**, 162 (1966).
CARLSON, L. A., EKELUND, L. G., ORÖ, L.: Clinical and metabolic effects of different doses of prostaglandin E_1 in man. Acta med. scand. **183**, 423 (1968).
COREY, E. J., ANDERSEN, N. H., CARLSON, R. M., PAUST, J., VEDEJS, E., VLATTAS, I., WINTER, R. E. K.: Total synthesis of prostaglandins. Synthesis of the pure dl—E_1, —$F_{1\alpha}$, —$F_{1\beta}$, —A_1 and —B_1 hormones. J. Amer. chem. Soc. **90**, 3245 (1968).
Editorial: Prostaglandin. New Engl. J. Med. **277**, 1091 (1967).
EMBREY, M. P.: Induction of abortion by prostaglandins E_1 and E_2. Brit. med. J. **1970II**, 258.
EULER, U. S. VON, ELIASSON, R.: Prostaglandins. New York/London: Academic Press 1967.
HIGGINS, CH. B., BRAUNWALD, E.: The prostaglandins. Biochemical, physiologic and clinical considerations. Amer. J. Med. **53**, 92 (1972).
HORTON, E. W.: Prostaglandins. Monographs on endocrinology, Vol. 7. Berlin-Heidelberg-New York: Springer 1972.
KARIM, S. M. M.: Use of prostaglandin E_2 in the management of missed abortion, missed labour and hydatidiform mole Brit. med. J. **1970 3**, 196.
— FILSHIE, G. M.: Therapeutic abortion using prostaglandin $F_{2\alpha}$. Lancet **1970I**, 157.
— — Use of prostaglandin E_2 for therapeutic abortion. Brit. med. J. **1970 3**, 198.
— — The prostaglandins. Oxford: MTP Medical and technical publishing Co. Ltd. 1972.
KIRTON, K. T., PHARRISS, B. B., FORBES, A. D.: Luteolytic effects of prostaglandin $F_{2\alpha}$ in primates. Proc. Soc. expt. Biol. (N. Y.) **133**, 314 (1970).
Leading article: Prostaglandins. Lancet **1968I**, 30.
— Prostaglandins and the induction of labour or abortion. Lancet **1970I**, 927.
LEE, J. B.: Antihypertensive activity of the kidney—the renomedullary prostaglandins. New Engl. J. Med. **277**, 1073 (1967).
MCCRACKEN, J. A., GLEW, M. E., SCARAMUZZI, R. J.: Corpus luteum regression induced by prostaglandin $F_{2\alpha}$ 1. J. clin. Endocr. **30**, 544 (1970).
PHARRISS, B. B., TILLSON, S. A., ERICKSON, R. R.: Prostaglandins in luteal function. Recent Progr. Hormone Res. **28**, 51 (1972).
RAISZ, L. G.: Prostaglandins and the hypercalcemia of cancer. New Engl. J. Med. **289**, 214 (1973).
RAMWELL, P. W. (ed.): Prostaglandins, vol. 1. New York-London: Plenum Press 1973.
— SHAW, J. E.: Biological significance of the prostaglandins. Recent Progr. Hormone Res. **26**, 139 (1970).
— — (eds.): Prostaglandins. Ann. N. Y. Acad. Sci. **180** (1971).
SAMUELSSON, B.: Biosynthesis and metabolism of prostaglandins. Progr. biochem. Pharmacol. **3**, 59 (1967).
SANDLER, M., KARIM, S. M. M., WILLIAMS, E. D.: Prostaglandins in amine-peptide-secreting tumours. Lancet **1968II**, 1053.
SPEROFF, L., RAMWELL, P. W.: Prostaglandin stimulation of in vitro progesterone synthesis. J. clin. Endocr. **30**, 345 (1970).
WERNING, C., SIEGENTHALER, W.: Prostaglandine und Niere. Dtsch. med. Wschr. **95**, 2345 (1970).
WILLIAMS, E. D., KARIM, S. M. M., SANDLER, M.: Prostaglandin secretion by medullary carcinoma of the thyroid. Lancet **1968I**, 22.

Added in Proofs:

DAVIS, Z., MOERTEL, C. G., MCILRATH, D. C.: The malignant carcinoid syndrome. Surg. Gynec. Obstet. **137**, 637 (1973).

XVI. Endocrine Hyperfunctional Syndromes by Ectopic Hormone Formation (Paraneoplastic Syndromes)

A. LABHART

A. Definition

Ectopic formation of hormones means the production of most probably normal hormones by tumors of tissues which normally form no hormones or different hormones. These tumors are with few exceptions malignant.

The term "ectopic hormone formation" is preferable to the word "paraneoplasia" since the term paraneoplastic syndrome includes both hormonal and non-hormonal distant effects of malignant tumors, such as neuropathy, myopathy, angiopathy, blood disorders and dermatoses.

At present, 15 syndromes caused by overproduction of ectopically formed hormones are known. In 11 of these syndromes, protein and polypeptide hormones, which are otherwise produced by endocrine glands, are formed ectopically. Mostly they are of polypeptide nature formed in malignant tumors of tissues which are of ectodermal origin (neutral crest) or arise from entodermal tissue (WEICHERT, 1970). It is striking that so far no ectopic steroid formation which stems from tissues of neurodermal origin has been discovered (Table 1 after LIPSETT, 1964).

Relations between malignant tumors and endocrine diseases have been known since 1928, when BROWN first described what was apparently CUSHING'S syndrome associated with a bronchus carcinoma. The fact that malignant tumors produce hormones became obvious when it was observed that the endocrinopathy disappeared after the removal of the tumor and reappeared with the development of metastases. LIDDLE

Table 1. Hyperfunctional syndromes caused by ectopically formed hormones

Syndrome	Demonstration of hormone	
	in blood	in tumor
Glandular polypeptide hormones		
Cushing's syndrome	ACTH↗	ACTH, MSH
Cushing's syndrome		CRF
Hyperparathyroidism	Parathormone↗	Parathormone
Schwartz-Bartter syndrome	Vasopressin↗	Vasopressin
Hyperthyroidism	TSH↗	TSH
Acromegaloid / Osteoarthropathy	STH↗	
Precocious puberty / Gynecomastia	FSH↗, LH↗	FSH, LH
Gynecomastia	Placental lactogen↗ Chorionic gonadotropin↗	Placental lactogen Chorionic gonadotropin
Hypoglycemia	Insulin (IRI)↗	Insulin (IRI)
Galactorrhea	Prolactin↗	Prolactin
Constipation / Steatorrhea	Glucagon	Glucagon
Tissue hormones		
Polycythemia	Erythropoietin↗	Erythropoietin
Carcinoid with bronchus CA	Serotonin↗ ? Bradykinin↗	Serotonin? Bradykinin
Zollinger-Ellison syndrome	Gastrin↗	Gastrin
Medullary Ca of the thyroid gland	ACTH↗	Calcitonin Prostaglandin
Similar syndrome produced by non-endocrine humoral substances		
Hypoglycemia in large tumors		Tryptophane? Niacin?

(1961), CHRISTY (1961) and HOLUB (1961) have succeeded in demonstrating ACTH in tumors and in the plasma of patients with paraneoplastic Cushing's syndrome. It is now almost certain that the ectopically-formed ACTH is identical to pituitary ACTH, since they both have the same chemical, physical, biological and immunological properties as far as can be determined by present-day methods. Since then, vasopressin, a parathormone-like polypeptide, FSH, LH, TSH, STH, erythropoietin, serotonin, gastrin, and prostaglandin have been successfully demonstrated in the blood, in tumors, or in both. It is, however, not certain whether these latter hormones formed ectopically are chemically identical to the corresponding glandular hormones. One tumor can produce a very wide variety of hormones (O'NEAL, 1968), and different tumors may form the same hormones.

The hormone-producing tumors are almost always malignant. The oat-cell bronchus carcinoma is especially common, but other tumors can be responsible: squamous-cell bronchus carcinoma, carcinoma of the thymus, islet-cell carcinomas, adrenocortical carcinomas, hypernephromas, adenocarcinomas of the gut, hepatomas, tumors of the uterus, prostate and nervous tissue. Benign tumors have been observed to form erythropoietin.

B. Pathogenesis

The question of how the undifferentiated cancer cell with a defective genetic system, a disturbed metabolism, and uncontrolled growth can complete complicated syntheses of polypeptides with molecular weights from 176 (serotonin) to over 30000 (gonadotropin) is one of the most fascinating problems in endocrinology, oncology, and genetics.

Since hormones have been successfully extracted from the tumors, numerous earlier hypotheses such as the predisposition of endocrinopathies to tumors or of neoplasia to endocrinopathies have been discarded. The view that the tumors only represent a sort of sponge for the hormones which stores normally formed hormones and protects them from degradation can be excluded by the duration of the illness and the high production rates which can be demonstrated for certain hormones formed ectopically. The argument that it is possible that a few of the many constellations of the amino-acid compounds developed within the abnormal metabolism and abnormal growth of the cancer cell could accidentally correspond to polypeptide hormones (BOWERS, 1966), is easily refuted by application of the law of probability. There is an extremely large number of possible variations for the arrangement of the amino acids of a protein hormone such as FSH with a molecular weight of 30000.

The hypothesis put forward by LIPSETT (1965) now seems the most probable: Every cell of the body is probably provided with the same genetic code, so that presumably each possesses the information necessary to synthesize any protein. Under normal conditions, most of this information is repressed. In the neoplastic variation of the cell, malignant or benign, derepression occasionally occurs, which is not normal in postnatal life (WALDENSTRÖM, 1970). This process could also be compared to a dedifferentiation, where there is a loss of the specific control mechanisms which prevent every cell from using all the information stored in its DNA. Derepression of protein-forming templates only active in the fetus have been observed, such as the formation of fetuin in hepatomas.

The clinical pictures of the hyperfunctional syndromes of ectopic hormones are dealt with in the appropriate chapters. They may differ in several aspects from the usual endocrine disease, as in Cushing's syndrome (see Chap. VII). We refrain from discussing there the syndrome of severe and often fatal hypoglycemia associated with large, usually retroperitoneal, tumors. It was not possible to demonstrate any insulin or insulin-like activity in the plasma of the patients or in the tumors of the majority of well over 100 cases reported. The demonstration of an insulin-like activity with the diaphragm of the rat or by the epididymal fatty tissue methods can only be accepted as specific if it can be inhibited with insulin antibodies. Hyperinsulinism has been demonstrated immunologically only twice (OLEESKY, 1962; SHAMES, 1968). In the tumors themselves it has only once been possible to detect immunologically reactive insulin. However, with the fat-pad method no more insulin-like activity could be detected than in other tissues. It is improbable that these tumors, which are always very large, produce insulin. On the other hand, they appear to block glycogenolysis, gluconeogenesis and lipolysis through humoral substances (JAKOB, 1967; FROESCH, 1968) (see p. 818).

Only the cases of hypercalcemia and *hypo*phosphatemia can probably be included in the syndrome of the ectopic formation of parathormone. Increased amounts of vitamin D or similar substances cannot be demonstrated in the blood or in the tumors in cases with hypercalcemia and *hyper*phosphatemia which are often seen with bone metastases and can be normalized by cortisone application. Increased production of

gonadotropins with endocrine syndromes (pubertas praecox, gynecomastia) can only be attributed to the syndrome of ectopic hormone production if these gonadotropins are not produced by tumors of the placenta or of trophoblastic tissue. Increased amounts of FSH and LH have been demonstrated in the blood and tumors of patients with bronchus carcinomas (see p. 488). The excretion of gonadotropin and estrogen is increased (BECKER, 1968; ROSEN, 1968), and strangely, osteoarthropathy of unknown pathogenesis usually exists at the same time. Galactorrhea can result form prolactin production in malignant tumors (TURKINGTON, 1971). In one case of osteoarthropathy with a bronchus carcinoma, in the absence of gynecomastia the growth hormone concentration in the blood was found to be raised. This returned to normal with the disappearance of the joint complaints after removal of the tumor (STEINER, 1968). Growth hormone can be synthesized by cell cultures of lung carcinoma (GREENBERG, 1972).

By the strictest definition, the Zollinger-Ellison syndrome is due to the ectopic formation of gastrin. However, since the pancreas might be a potential producer of gastrin under pathological conditions (see p. 973), only the atypical forms which do not develop from an islet-cell adenoma are to be considered as the paraneoplastic syndrome. The carcinoid syndrome (see p. 984) can only be considered as paraneoplasia in its atypical forms or if combined with production of other ectopic hormones.

The therapy can only be causal and involves the surgical removal of the hormone-active tumor. Not uncommonly, the endocrine illness can cause the tumor to be detected relatively early (gynecomastia in heavy smokers). When the tumors are inoperable or have metastasized, radiation and cytostatics must be considered. Removal of the end-organ is only justified in exceptional cases such as in the Zollinger-Ellison syndrome.

References

Ectopic Hormones

AZZOPARDI, J.G., WILLIAMS, D.D.: Pathology of nonendocrine tumors associated with Cushing's syndrome. Cancer (Philad.) **22**, 274 (1968).

BECKER, K.L., COTTRELL, J., MOORE, CH.F., WINNACKER, J.L., MATTHEWS, M.J., KATZ, S.: Endocrine studies in a patient with a gonadotropin-secreting bronchogenic carcinoma. J. clin. Endocr. **28**, 809 (1968).

BOWERS, B.F., GORDAN, G.S.: Hormonal effects of nonendocrine tumors. Ann. Rev. Med. **16**, 83 (1965).

BROWN, W.H.: Case of pluriglandular syndrome; "diabetes of bearded women". Lancet **1928 II**, 1022.

CHRISTY, N.P.: Adrenocorticotrophic activity in the plasma of patients with Cushing's syndrome associated with pulmonary neoplasms. Lancet **1961 I**, 85.

DONAHOWER, G.F., SCHUMACHER, O.B., HAZARD, J.B.: Medullary carcinoma of the thyroid—a cause of Cushing's syndrome—report of two cases. J. clin. Endocr. **28**, 1199 (1968).

FAIMAN, CH., COLWELL, J.A., RYAN, R.J., HERSHMAN, J.M., SHIELDS, TH. W.: Gonadotropin secretion from a bronchogenic carcinoma. New Engl. J. Med. **277**, 1395 (1967).

FROESCH, E.R., JACOB, A., LABHART, A.: Hypoglykämie bei extrapankrealen Tumoren. In: Nebenschilddrüse und endokrine Regulation des Calciumstoffwechsels. Spontanhypoglykämie Glucagon (Ed. by KRACHT, J.). Berlin-Heidelberg-New York: Springer 1968.

FUSCO, F.D., ROSEN, S.W.: Gonadotropin-producing anaplastic large-cell carcinomas of the lung. New Engl. J. Med. **275**, 507 (1966).

GARDNER, B.: Hypercalcemia and malignancy. J. Amer. med. Ass. **204**, 24 (1968).

GINSBURG, J., BROWN, J.B.: Increased oestrogen excretion in hypertrophic pulmonary osteoarthropathy. Lancet **1961 II**, 1274.

GLEESON, M.H., BLOOM, S.R., POLAK, J.M., HENRY, K., DOWLING, R.H.: Endocrine tumor in kidney affecting small bowel structure, motility and absorptive function. Gut **12**, 773 (1971).

GORDAN, G.S., FITZPATRICK, N.E., LUBITCH, W.P.: Identification of osteolytic sterols in human breast cancer. Trans. Ass. Amer. Phycns **80**, 183 (1967).

GREENBERG, P.B., BECK, C., MARTIN, T.J., BURGER, H.G.: Synthesis and release of human growth hormone from lung carcinoma in cell culture. Lancet **1972 I**, 350.

HAAS, H.G.: Endokrine Überfunktion bei malignen Tumoren. Praxis **18**, 610 (1964).

HUGUENIN, R., FAUVET, J., PIÉRAT, A., LÉOBARDY, J. DE: Le syndrome «cancer bronchopulmonaire, périostite des os longs, gynécomastie». Bull. Soc. méd. Hôp. Paris **70**, 986 (1954).

JAKOB, A., MEYER, U.A., FLURY, R., ZIEGLER, W.H., LABHART, A., FROESCH, E.R.: The pathogenesis of tumor hypoglycemia: Blocks of hepatic glucose release and of adipose tissue lipolysis. Diabetologia **3**, 506 (1967).

JONES, J.E., SHANE, S.R., GILBERT, E., FLINK, E.B.: Cushing's syndrome induced by the ectopic production of ACTH by a bronchial carcinoid. J. clin. Endocr. **29**, 1 (1969).

KATZ, A., KAPLAN, L., MASSRY, S.G., HELLER, R., PLOTKIN, D., KNIGHT, I.: Primary hyperparathyroidism in patients with breast carcinoma.

KRACHT, J.: Pathologie der ektopisch hormonbildenden Tumoren. Verh. dtsch. Ges. inn. Med. **73**, 488 (1967).

LABHART, A.: Endokrine Überfunktionssyndrome der Gewebehormone. In: Gewebe- und Neurohormone. Physiologie des Melanophorenhormons. 8. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1962.

LAW, D.H., LIDDLE, G.W., SCOTT, H.W., JR., TAUBER, ST.D.: Ectopic production of multiple hormones (ACTH, MSH and Gastrin) by a single malignant tumor. New Engl. J. Med. **273**, 292 (1965).

LIDDLE, G.W., GIVENS, J.R., NICHOLSON, W.E., ISLAND, D.P.: The ectopic ACTH syndrome. Cancer Res. **25**, 1057 (1965).

— NICHOLSON, W.E., ISLAND, D.P., ORTH, D.N., ABE, K., LOWDER, S.C.: Clinical and laboratory studies of ectopic humoral syndromes. Recent Progr. Hormone Res. **25**, 283 (1969).

LIPSETT, M.B.: Hormonal syndromes associated with neoplasia. Advances in metabol. disorders **3**, 112 (1968).

— ODELL, W.D., ROSENBERG, L.E., WALDMANN, TH.A.: Humoral syndromes associated with nonendocrine tumors. Ann. intern. Med. **61**, 733 (1964).

O'NEAL, L.W.: Secretion of various endocrine substances by ACTH-secreting tumors: gastrin, melanotropin, norepinephrin, serotonin, parathormone, vasopressin, glucagon. Cancer (Philad.) **21**, 1219 (1968).

ROSEN, S.W., BECKER, CH.E., SCHLAFF, S., EASTON, J., GLUCK, M.C.: Ectopic gonadotropin production before clinical recognition of bronchogenic carcinoma. New Engl. J. Med. **279**, 640 (1968).

ROSS, E.J.: The cancer cell as an endocrine organ. In: James, Recent advances in endocrinology, 8th ed. London: Churchill 1968.

— Endocrine and metabolic manifestations of Cancer. Brit. med. J. **1972I**, 735.

SANDLER, M., KARIM, S.M.M., WILLIAMS, E.D.: Prostaglandins in amine-peptide-secreting tumors. Lancet **1968II**, 1053.

SPARAGANA, M., PHILLIPS, G., HOFFMAN, C., KUCERA, L.: Ectopic growth hormone syndrome associated with lung cancer. Metabolism **20**, 730 (1971).

STEINER, H., DAHLBÄCK, O., WALDENSTRÖM, J.: Ectopic growth hormone production and osteoarthropathy in carcinoma of the bronchus. Lancet **1968I**, 783.

STROTT, CH.A., NUGENT, CH.A., TYLER, F.H.: Cushing's syndrome caused by bronchial adenomas. Amer. J. Med. **44**, 97 (1968).

STUDER, H., STAUB, J.J., WYSS, F.: Klinische und metabolische Fernwirkungen maligner Tumoren. Schweiz. med. Wschr. **101**, 446 (1971).

TAMM, J.: Die Klinik paraneoplastischer Endokrinopathien. Verh. dtsch. Ges. inn. Med. **73**, 481 (1967).

TURKINGTON, R.W.: Ectopic production of prolactin. New Engl. J. Med. **285**, 1455 (1971).

UPTON, G.V., AMATRUDA, TH.T, JR.: Evidence for the presence of tumor peptides with corticotropin releasing factor-like activity in the ectopic ACTH syndrome. New Engl. J. Med. **285**, 419 (1971).

VORHERR, H., MASSRY, S.G., UTIGER, R.D., KLEEMAN, C.R.: Antidiuretic principle in malignant tumor extracts from patients with inappropriate ADH syndrome. J. clin. Endocr. **28**, 162 (1968).

WALDENSTRÖM, J.: Maladies of derepression. Schweiz. med. Wschr. **100**, 2197 (1970).

WEICHERT, R.F.: The neural ectodermal origin of the peptide-secreting endocrine glands. Amer. J. Med. **49**, 232 (1970).

WEINTRAUB, B.D., ROSEN, S.W.: Ectopic production of human somatomammotrophin by nontrophoblastic cancers. J. clin. Endocr. **32**, 94 (1971).

WILLIAMS, E.D., KARIM, S.M.M., SANDLER, M.: Prostaglandin secretion by medullary carcinoma of the thyroid. Lancet **1968I**, 22.

XVII. Thymus

P. J. GROB and A. LABHART

Studies over the past years have established that the thymus has a central role in the immune system (MILLER, 1969; METCALF, 1966; HESS, 1968; BURNET, 1962). Evidence has been found that the thymus might exert at least part of its function by acting as an endocrine organ. Recent findings also suggest that thymic hormones might influence the neuromuscular function (GOLDSTEIN, 1971; WHITE, 1970).

1. Embryology and Structure

Embryologically the thymus arises from an epithelial outgrowth of the third and the fourth branchial pouches, which later forms a sponge-like structure. In fetal life the human thymus becomes predominantly lymphoid and this histological pattern is preserved throughout childhood. Thereafter, the lymphoid component gradually decreases. The human thymus accounts for approximately 0.8% of the body weight at birth. Its relative size then gradually decreases. Although it has not been fully established, many data suggest that the lymphoid cells within the thymus originate from stem cells of bone marrow or related organs (FORD, 1966). They divide within the thymus, and most of them die in situ while others seem to go on to populate the peripheral lymphatic organs.

The histological units of the thymus are lobules, which are joined in the center of the organ. The cortical areas of the lobules are packed with predominantly small lymphocytes, which show a high rate of mitoses and cell death. These cells are highly sensitive to X-ray irradiation. In contrast to peripheral lymph nodes, no germinal centers are usually seen. In the medullar region, on the other hand, where the epithelial mesh work is densest, relatively few lymphocytes are present which do not divide and are not sensitive to X-ray irradiation. Epithelial cells are aggregated into whorled patterns (the Hassall's corpuscles). Myoid cells are also found.

2. Thymus and Immune Function

A normally functioning thymus is necessary for the development and expression of at least part of lymphoid tissue and its immunological function (MILLER, 1969; ROITT, 1969): lymphoid stem cells appear to differentiate and acquire immunologic competence under its influence. Most of the evidence collected points to two possible modes of thymic action: a) Lymphoid stem cells migrate into the thymus where they mature and attain immune reactivity by direct contact in situ within the gland. b) Thymic hormones, such as thymosine and others, act locally and/or peripherally to endow lymphoid cells with the capacity to respond to antigenic stimuli. Evidence for the endocrine hypothesis derives from experiments in which neonatally thymectomized mice were reconstituted with cell-free thymic extracts or with thymic tissue placed in cell-impermeable Millipore chambers (OSOBA, 1963). Both mechanisms (a and b) could act cooperatively or additively.

An immunoactive thymic extract called thymosine was described by GOLDSTEIN and collaborators in 1966. Further investigations have suggested that it might be a protein influencing the rate of maturation of immunological competence in more primitive potentially competent lymphoid cells. Further evidence of immunoactive factors has been found by COMSA (1955), TRAININ (1970), BACH (1971), DUTTON (1971) and others.

While the thymus plays a major role in the ontogenesis of the immune system, its influence in adult life is less clear. It may act as a supply organ, functioning predominantly when the peripheral lymphoid system is exhausted.

The immune system is divided into cell-mediated (cellular immune response) and antibody-mediated phenomena (humoral immune response). T cells (mediators of cellular immunity) seem to derive from and be under the control of the thymus, while the B cells (mediators of the humoral immune response) seem to be less dependent on its influence. On the other hand, T cells have recently been found to amplify the B-cell function by a "helper"

mechanism (cell cooperation). Thus, the thymus may modulate the B cell-mediated reactions at least indirectly. (Its influence is necessary for primary antibody production against certain antigens.) It is also possible that there are various subpopulations of T cells (RAFF, 1971).

Our knowledge of thymic function and of the consequences of defects is based mainly on observations in animal experiments. At least some conditions point to similar situations in man: Congenital absence or dysfunction of the thymus in the human, as for example in infants with DiGeorge syndrome, is characterized by an almost total absence of cell-mediated immune responses, causing conditions such as lymphopenia and a marked susceptibility to a wide variety of infectious agents, particularly of viral or fungal nature (DIGEORGE, 1968). Transplants are not rejected, and delayed skin reactions cannot be elicited. In contrast, only inconsistent antibody deficiencies are observed. Patients with DiGeorge syndrome have been successfully treated with thymic transplants (AUGUST, 1968; CLEVELAND, 1968). There are other human diseases associated with thymic abnormalities and partial or total defects of cellular immune functions, such as reticular dysgenesis, alymphocytic agammaglobulinemia (Swiss type), ataxia telangiectatica and other combined immune deficiencies (NEZELOF, 1964; ROGERS, 1968; GABRIELSEN, 1969).

SORKIN *et al.* (SORKIN, 1970; PIERAPOLI, 1969; FABRIS, 1972) have found evidence that thymic function itself might be under the control of the adenohypophysis. These authors also emphasize the possible importance of the thymus as a biological clock and of hormones in the aging processes of lymphocytes and in aging as such. Their evidence is based mainly on work with hypopituitary dwarf mice.

3. Thymus and Neuromuscular Function

The frequent association of myasthenia gravis and thymic abnormalities is well known. Thymectomy may be of value in patients with myasthenia gravis (PAPATESTAS, 1971). Recently, GOLDSTEIN *et al.* have found experimental evidence for two different biologically active extracts of thymic origin, "thymotoxin", a basic polypeptide which induces myositis, and "thymin", which leads to a neuromuscular block similar to that seen in myasthenic patients when given by injection in rat (GOLDSTEIN, 1971).

4. Other Thymic Hormones

Other hormone-like agents have been attributed to the thymus, as well as thymosin and related substances. These include promin, which promotes growth, and retin (SZENT-GYÖRGY, 1962), which is thought to inhibit growth and fertility. The thymus extract thymocrescin (Asher) is also thought to promote growth. Another substance, infertin, has been isolated from promin and is believed to induce sterility in rats, temporarily in adult and permanently in young animals. These substances have so far neither been sufficiently characterized to qualify as hormones, nor is their nature or mode of action clearly understood.

References

Thymus

AUGUST, C.S., ROSEN, F.S., FILLER, R.M., JANEWAY, C.A., MARKOWSKI, B., KAY, H.E.M.: Implantation of a fetal thymus restoring immunological competence in a patient with thymic aplasia (Di George's syndrome). Lancet **1968 II**, 1210.

BACH, J.-F., DARDENNE, M., GOLDSTEIN, A.L., GUHA, A., WHITE, A.: Appearance of T-cell markers in bone marrow rosette-forming cells after incubation with thymosin, a thymic hormone. Proc. nat. Acad. Sci. (Wash.) **68**, 2734 (1971).

BURNET, M.: The thymus gland. Sci. Amer. **207**, 50 (1962).

CLEVELAND, W.W., FOGEL, B.J., BROWN, W.T., KAY, H.E.M.: Fetal thymic transplant in a case of Di George's syndrome. Lancet **1968 II**, 1211.

COMSA, J.: Influence of a highly purified thymus extract on the leucopoiesis in guinea-pigs. Acta endocr. (Kbh.) **19**, 406 (1955).

DIGEORGE, A.M.: Congenital absence of the thymus and its immunologic consequences: Concurrence with congenital hypoparathyroidism. Birth defects **4**, 116 (1968).

DUTTON, R.W., FALKOFF, R., HIRST, J.A., HOFFMANN, M., KAPPLER, J.W., KETTMAN, J.R., LESLEY, J.F., VANN, D.: Is there evidence for a non-antigen specific diffusable chemical mediator from the thymus-derived cell in the initiation of the immune response? In: Progress in immunology (ed.: B. AMOS), First International Congress of Immunology, p. 355—368. New York: Academic Press 1971.

FABRIS, N., PIERAPOLI, W., SORKIN, E.: Lymphocytes, hormones and aging. Nature (Lond.) **240**, 557 (1972).

FORD, C.E.: Traffic of lymphoid cells in the body in the thymus: Experimental and clinical studies. Ciba Foundation Symposium. London: Churchill 1966.

GABRIELSEN, A.E., COOPER, M.D., PETERSON, R.D.A., GOOD, R.A.: The primary immunologic deficiency diseases. In: Textbook of immunopathology, vol. II (eds.: P.A. MIESCHER and H.J. MULLER-EBERHARD, p. 385—405. New York-London: Grune & Stratton 1969.

GOLDSTEIN, A.L., SLATER, F.D., WHITE, A.: Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). Proc. nat. Acad. Sci. (Wash.) **56**, 1010 (1966).

GOLDSTEIN, G.: Myasthenia gravis and the thymus. Ann. Rev. Med. **22**, 119 (1971).

— HOFMANN, W.W.: Experimental myasthenia gravis. Res. Publ. Ass. nerv. ment. Dis. **49**, 241 (1971).

HESS, M.W.: Experimental thymectomy. Possibilities and limitations, p. 105. Berlin-Heidelberg-New York: Springer 1968.

METCALF, D.: The thymus. In: Recent results in cancer research, vol. 5, p. 144. Berlin-Heidelberg-New York: Springer 1966.

MILLER, J. F. A. P.: The thymus and the development of immunologic responsiveness. Science **144**, 1544 (1964).

— MITCHELL, G. F.: Thymus and antigen-reactive cells. Transplant. Rev. **1**, 3 (1969).

NEZELOF, C., JANNET, M. L., LORTHOLARY, P., LABRUNE, H., LAMY, J.: L'hypoplasie héréditaire du thymus. Arch. franç. Pédiat. **21**, 897, 920 (1964).

OSOBA, D., MILLER, J. F. A. P.: Evidence for a humoral thymus factor responsible for the maturation of immunological faculty. Nature (Lond.) **199**, 653 (1963).

PAPATESTAS, A. E., ALPERT, L. I., OSSERMAN, K. E., OSSERMAN, R. S., KARK, A. E.: Studies in myasthenia gravis: Effects of thymectomy. Results on 185 patients with nonthymomatous and thymomatous myasthenia gravis, 1941—1969. Amer. J. Med. **50**, 465 (1971).

PIERAPOLI, W., SORKIN, E.: Relationship between developmental hormones, the thymus, and immunological capacity. In: Lymphatic tissue and germinal centers in immune response, p. 397—401. New York: Plenum Press 1969.

RAFF, M. C., CANTOR, H.: Subpopulations of thymus cells and thymus-derived lymphocytes. In: Progress in immunology (ed.: B. AMOS), First International Congress of Immunology, p. 83—93. New York: Academic Press 1971.

ROGERS, B. H. G., MANALIGOD, J. R., BLAZEK, W. V.: Thymoma associated with pancytopenia and hypogammaglobulinemia. Amer. J. Med. **44**, 154 (1968).

ROITT, I. M., GREAVES, M. F., TORRIGIANI, G., BROSTOFF, J., PLAYFAIR, J. H. L.: The cellular basis of immunological response. A synthesis of some current views. Lancet **1969 II**, 367.

SORKIN, E., PIERAPOLI, W., FABRIS, N., BIANCHI, E.: Relation of growth hormone to thymus and the immune response. In: Growth and growth hormone (eds.: A. PECILE and E. E. MUELLER), p. 132. Amsterdam: Excerpta Medica 1972.

SZENT-GYÖRGYI, A., HEGYELI, A., MCLAUGHLIN, J. A.: Constituents of the thymus gland and their relation to growth fertility, muscle and cancer. Proc. nat. Acad. Sci. (Wash.) **48**, 1439 (1962).

TRAININ, N., SMALL, M.: Conferment of immunocompetence on lymphoid cells by a thymic humoral factor. In: Hormones and the immune response. CIBA Foundation Study Group No. 36 (eds.: G. E. W. WOLSTENHOLME and J. KNIGHT), p. 24—41. London: J. & A. Churchill 1970.

WHITE, A., GOLDSTEIN, A. L.: Thymosin, a thymic hormone influencing lymphoid cell immunological competence. In: Hormones and the immune response. CIBA Foundation Study Group No. 36 (eds.: G. E. W. WOLSTENHOLME and J. KNIGHT), p. 3—23. London: J. & A. Churchill 1970.

XVIII. The Pluriglandular Syndromes

A. LABHART

A. Endocrine Adenomatoses

The rare hereditary syndromes of endocrine adenomatosis have been known to pathologists since the turn of the century (ERDHEIM, 1903). Clinicians have been able to diagnose the syndrome for the past 30 years (ROSSIER, 1939; SHELBOURNE, 1945; UNDERDAHL, 1953; WERMER, 1954), and about 100 cases have now been described (BALLARD, 1964; JOHNSON, 1967). There is a hypothesis concerning the pathogenesis of these syndromes which also takes into account the occasional occurence of ectopic peptide hormones in certain types of endocrine adenomatosis (WEICHERT, 1970). It is proposed that neuroendocrine cells migrate to the primitive gastrointestinal tract from where they form the endocrine cells of the anterior pituitary and the parathyroid glands and islet cells of the pancreas. Neuroendocrine cells, as argentaffin cells, are scattered throughout the gastrointestinal tract mucosa and also occur in other organs such as the bronchi. Related cells can be found in the adrenal medulla. These neuroendocrine cells may occasionally develop in the tumors producing peptide hormones. Thus, the syndrome of endocrine adenomatosis may be a dysplasia of neural ectoderm. Cytochemical methods make it possible to distinguish a series of cells stemming from the primitive alimentary tract and maturing into endocrine cells of the anterior pituitary, the pancreas islet α_2- and β-cells and the c-cells of the thyroid. On the other hand, they form the enterochromaffine cells of the gut and argyrophil cells of the stomach. They all have the ability to form peptide hormones and have been called cells of the APUD-series (amine-precursor-uptake and decarboxylation (PEARSE, 1968).

There are two distinct types of endocrine adenomatosis; one is characterized by benign tumors of the parathyroids and pituitary, and benign or malignant growths in the islet cells of the pancreas, and the other by medullary carcinoma of the thyroid and bilateral pheochromocytoma, the medullary carcinoma producing ectopically formed hormones in addition to calcitonin.

1. Multiple Endocrine Adenomatosis (Endocrine Polyadenomatosis, Familial Multiple Endocrine Adenoma (MEA), Wermer's Syndrome, "Job's Syndrome", Steiner's Multiple Neoplasia,Type I)

This syndrome is centralized around benign tumors of the parathyroids and adenohypophysis, and benign or malignant growths in the islet cells of the pancreas. Corresponding endocrine hyperfunctional or deficiency syndromes are also present; in addition, the majority of patients suffer from peptic ulcers. The fully developed Zollinger-Ellison syndrome may sometimes be present, or sometimes there is a tendency to recurrent and often multiple gastric and duodenal ulcers.

The tumors and hyperplasia occasionally observed in the thyroid gland, adrenal medulla and cortex and testes probably do not belong to this syndrome. The illness can become manifest at any age and the frequency is the same in both sexes. The disease begins as hyperfunction of one gland and can be followed by hyperfunction in other glands and/or pituitary failure. Every combination is possible.

The parathyroids are most frequently affected (80–90%). Functioning adenomas are usually found in several glands and there may be several in each of the parathyroids. Patho-anatomical examination reveals hyperplasia of the chief cells, chief cell adenomatosis or adenomas (see p. 908). A striking feature is the benign course of hyperparathyroidism over years and sometimes even decades with only slight hypercalcemia. Renal stones and nephrocalcinosis occur significantly less frequently than in ordinary hyperparathyroidism. Skeletal involvement is uncommon, and hypercalcemia is often an incidental finding. This and the multiplicity of the adenomas indicates reservation in surgical treatment, which is only justified if the patient complains or if complications appear likely. Total resection of the three largest glands and subtotal resection of the smallest is usually necessary in such cases, and the latter should be marked in case another operation is required (see p. 932).

Recent reviews suggest that tumors of the islet cells are found in 45–80% of cases and are nearly always multiple. Adenomas or carcinomas (multiple) of the non-B-cells are found in over half the cases. About one third of the cases are affected by multiple B-cell adenomas and occasionally by B-cell carcinomas. Solitary adenomas and different combinations are found in the remaining cases.

In a third of the cases there is hyperinsulinism with hypoglycemic attacks requiring exploratory surgery and usually subtotal resection of the pancreas because of the frequent multiplicity of the adenomas. Non-B-cell adenomas or carcinomas cause the Zollinger-Ellison syndrome (see Chap. XV), which typically occurs in one fifth of multiple endocrine adenomatoses and is familial, probably developing through the same gene. On the other hand, they also cause the syndrome of pancreatogenic diarrhea ("pancreatic cholera", WDHA-syndrome), a severe illness with massive watery diarrhea and a heavy loss of potassium (see Chap. XV, p. 974), which is found in only about one eighth of cases.

Apart from the Zollinger-Ellison syndrome, over half of the patients with endocrine adenomatosis suffer from peptic ulcers which are more often multiple than solitary. The relationship of endocrine adenomatosis to "ordinary" ulceration is, however, not yet clear. Increased numbers of parietal cells are found in the gastric mucosa (MIEHER, 1962) in subjects with endocrine adenomatosis as well as in subjects with duodenal ulcers, and it is possible that this parietal cell hyperplasia has the same genetic basis or is a result of gastrin overproduction. The recognized frequency of the peptic ulcer in hyperparathyroidism (see p. 919) cannot entirely explain the nature of the relationship since the peptic ulcer occurs much more frequently in multiple endocrine adenomatosis than in hyperparathyroidism. Finally, increased secretion of gastrin or the interaction of different factors must be considered even if there is no evidence of non-B-cell tumors.

See p. 974 for the difficulties inherent in the therapy of the Zollinger-Ellison syndrome and pancreatogenic diarrhea.

Adenomas of the anterior pituitary occur in 65–70% of patients suffering from MEA. Almost half are chromophobe adenomas and one fourth are eosinophil adenomas associated with acromegaly. Chromophobe adenomas may be an incidental finding, or they may present the typical symptoms of loss in the visual fields and pituitary failure. Acromegaly in endocrine adenomatosis does not differ in any way from ordinary acromegaly. It is improbable that a basophil hyperplasia or a basophil adenoma are symptoms of this syndrome since Cushing's syndrome, which is radiosensitive was observed only once among 100 cases. A chromophobe adenoma was once observed with hyperthyreosis due to increased TSH production, acromegalia, and parathyroid adenoma (LAMBERG, 1969). See p. 122, 115 for the therapy of chromophobe and eosinophil adenomas. Conservative treatment must be selected in these cases too, because of other manifestations of the syndrome which are usually more important.

In addition, there appears to be a general predilection for developing tumors, and multiple lipomas, fibromas, sarcomas, polypoid adenomas of the gastrointestinal tract, meningiomas, and papillomas have been reported.

Observation of 12 families over 6 generations permitted the recognition of an autosomal dominant mode of inheritance with a high penetrance, variable expressivity and pleiotropy. The peculiarity of this very variable illness was usually repeated within one family. Sometimes up to half the affected members of the family were symptom-free (JOHNSON, 1967). However, multiple endocrine adenomatosis can be fatal, especially in combination with the fully developed Zollinger-Ellison syndrome. The ulcer condition with all its complications, particularly perforations, is the most common cause of death. Less frequently patients die from a unrecognized hypoglycemia or islet-cell carcinoma.

Life is particularly hard for those patients in whom one glandular disorder after another becomes manifest giving vital indications for repeated operations, and who see the suffering of other members of their family. This has led to the suggestion of naming the syndrome "JOB's" syndrome after the biblical sufferer (MOLDAWER, 1962).

Although this syndrome is rare, it is important to recognize it, since when the first manifestation is recognized as a part of multiple endocrine adenomatosis, it may be possible to institute purposeful life-saving surgical or substitution therapy, which may avoid the repeated, disappointing, and useless surgical interventions frequently recorded in the case histories of these patients (RENWICK, 1968).

The syndrome has also been observed in the dog (RUTISHAUSER, 1943) and can be produced in the rat by radiation (BERDJIS-CHAMSI, 1960).

2. Medullary Thyroid Carcinoma – Bilateral Pheochromocytoma (Sipple's Syndrome, Steiner's Multiple Endocrine Neoplasia, Type II)

Medullary carcinoma of the thyroid derives from the C- or parafollicular cells of the thyroid (see p. 232) which are the source of calcitonin.

The calcitonin concentration is grossly increased in the blood of patients with medullary carcinoma, which may be diagnostic for this tumor. Characteristically, this thyroid carcinoma also produces amyloid, which allows histological diagnosis. SIPPLE (1961) recognized the nonfortuitous association of medullary carcinoma with pheochromocytoma. In 60 to 80% of cases, the latter is bilateral. The syndrome is also transmitted by an autosomal dominant mode of inheritance with reduced penetrance (SAROSI, 1968). (Besides the hereditary form this disease can also occur sporadically.) In addition, there are often other malformations such as neurofibromatosis, intestinal ganglion neuromatosis, and neuromas of the mucosa, which appear mainly on the tongue and on the eyelids. The patients have a peculiar constitution reminiscent of the Marfan syndrome, with coarse features, thick lips, and chloasma-like pigmentations, especially around the mouth. In spite of the gross overproduction of calcitonin, the serum calcium of these patients is usually normal or only slightly below normal. However, very often there is hyperplasia or adenoma of the parathyroids. This might be due to a compensatory effect on chronic hypocalcemia or a direct stimulatory effect of calcitonin on the parathyroid cells (FISCHER, 1971).

If medullary carcinoma is diagnosed, all members of the patient's family should have the calcitonin content of their blood tested.

Diabetes mellitus is occasionally seen in these patients and may result from the inhibition of insulin release by the hormonal secretions of a pheochromocytoma.

Besides calcitonin, a medullary carcinoma is able to produce ACTH, MSH, prostaglandins, and serotonin. Thus Cushing's syndrome with various clinical characteristics is observed in about 3% of the patients with these syndromes. Bilateral adrenalectomy or necropsy always reveals marked bilateral hyperplasia of the adrenals (DONAHOWER, 1968).

About 30% of the patients suffer from diarrhea and sometimes steatorrhea. After removal of the tumor the diarrhea ceases. Prostaglandins have been found in high concentration in some medullary tumors; some prostaglandins are known to stimulate peristalsis. Serotonin has sometimes been detected, which can activate bradykinin and may explain the flush which occasionally occurs in these patients. Serum histaminase may be increased and can be inhibited by aminoguanidine.

For treatment of the hereditary type of medullary carcinoma, total thryoidectomy with removal of the regional lymph nodes is indicated as the carcinoma may be multicentric. Because of the relatively long benign course in the sporadic cases the operation can be restricted to the elimination of the tumor and resection of one lobe. Radiotherapy is probably beneficial. Radioiodine treatment is useless because there is no uptake by the tissue. Thyroxine medication should be given prophylactically. In any thyroidectomy for medullary carcinoma enlarged parathyroid glands should be looked for, even if the blood calcium level is normal. Pheochromocytoma and diabetes should also be ruled out.

For a review of familial medullary thyroid carcinoma and the syndrome of "familial chromaffinomatosis", based probably on an inherited defect of cells originating in the neural crest ["APUD" (PEARSE, 1971)], see MELVIN (1972).

It is still not definitely known whether there is a third syndrome of papillary thyroid carcinoma and adenomas of the parathyroids (ELLENBERG, 1962).

B. Autoimmune Polyendocrinopathy (Pluriglandular Insufficiency, Multiple Endocrine Sclerosis, Schmidt's Syndrome)

1. Definition

The syndrome of endocrine autoimmune polyadenopathy includes the primary disorder of two or more endocrine glands resulting, as a rule, in their insufficiency. The so-called primary or cytotoxic, non-tuberculous atrophy of the adrenal cortex is the main feature of the syndrome. It is most often combined with Hashimoto's thyroiditis or with hypothyroidism, and less commonly with hyperthyroidism. They are frequently associated with diabetes mellitus. Hypoparathyroidism may develop in addition to this triad. More often, however, it is found with primary adrenocortical atrophy combined only with moniliasis (see p. 895, 899). Primary failure of the gonads has also been observed. The pathogenesis can be complicated by the presence of "hypophysitis", a lymphocytic infiltration of the pituitary gland with or without fibrosis. When this arises, primary failure is theoretically possible in addition to the secondary. Finally, autoimmune diseases, in particular pernicious anemia, immunologically dependent steatorrhea, sporadic myasthenia gravis, and certain kinds of liver cirrhosis commonly occur with this syndrome. Occasionally striking alopecia is seen in women. All combinations are possible and most of these have been described. Table 1 gives the various frequencies. Over 150 cases of endocrine polyadenopathy have been detected so far. It shows a familial fre-

quency and the female sex is affected more often. Morphologically similar lymphocytic inflammations can be produced experimentally with organ extracts (LE COMPTE, 1966; LEVINE, 1967).

Table 1. Frequency of the combinations of the polyendocrinopathies. (After STEINER, 1968)

	Cases
Hypothyroidism + Addison's	102
Hypothyroidism + Addison's + Diabetes mellitus	32
Hypothyroidism + Addison's + Hypoparathyroidism	5
Hypothyroidism + Addison's + primary amenorrhea (2 d. m.)	8
Hypothyroidism + Diabetes mellitus	55
Addison's + Diabetes mellitus (also a few with adrenal TB)	125
Hypophysitis	3
Hypoparathyroidism + Addison's	22
Hypoparathyroidism + Hypothyroidism	?
Hypoparathyroidism + Diabetes mellitus	10
Hypoparathyroidism + Gonadal insufficiency	2
Hypoparathyroidism + Gonadal insufficiency + Addison's	1
(Hyperthyroidism + Addison's	68)

The syndrome was first described by CLAUDE and GOUGEROT in 1908 as "insuffisance pluriglandulaire". FALTA in 1912 suggested the term "multiple endocrine sclerosis" to emphasize the multiple, independent primary disorders of the endocrine system. Despite this, the term was frequently applied to classical cases of hypopituitarism. In 1926 SCHMIDT described the morbid anatomy of two cases of primary adrenocortical atrophy and a lymphomatous goiter with characteristic lymphocytic infiltration of the adrenals and thyroid gland. This infiltration can be clearly distinguished from the lymphocytic foci found in Addison's disease. Over the past ten years thyroid antibodies have successfully been demonstrated in Hashimoto's thyroiditis and in hypothyroidism (see p. 243, 152), adrenal antibodies in primary adrenocortical atrophy (see p. 313), antibodies to the gastric mucosa and the intrinsic factor in pernicious anemia, and to muscular tissue in myasthenia gravis; finally, antibodies to the parathyroids have been demonstrated in hypoparathyroidism (see p. 899). These antibodies can be detected in the blood in most, but not all, cases of endocrine polyadenopathy. Antibodies to the thyroid gland, the parathyroids, or the parietal cells of the gastric mucosa may be found in primary adrenocortical atrophy, even in the absence of demonstrable involvement of these organs. In any case, there is no correlation between the severity of the illness and the titer of the antibodies. It is worth mentioning that women with gonadal dysgenesia, and their relatives, often have antibodies to ovarian tissue, the thyroid gland, and the gastric mucosa (VALLOTON, 1967).

2. Pathogenesis

The demonstration of organ-specific antibodies and of chronic lymphocytic inflammation leading to atrophy strongly indicates immunological processes. The higher incidence in the female sex and the familial frequency of the syndrome also suggest immunological diseases. Nonetheless, no causal relation between the organ-specific antibodies and atrophy of the endocrine glands has been proven, and the question of the pathogenesis of this syndrome is still unanswered. It is hardly probable that the circulating antibodies alone are directly responsible for the lymphocytic infiltration and atrophy of the glands. The cellular immunological systems seem to be deranged, which may explain the lack of correlation between the severity of the disease and the antibody titer. The fact that no antibodies have been found in Addison's disease due to tuberculosis or other infections, however, makes it appear unlikely that the damaged glands liberate antigens and activate the immunological system. It is probable that these patients possess a familial, inherited disposition to autoimmune reactions, depending on a disturbed immune tolerance (IRVINE, 1967), and that the antibodies rather reflect a disturbed immunological system than that they cause it. Organspecific antibodies are also frequently found in healthy members of a patient's family (EVANS, 1967). Nevertheless, an indirect action of the antibodies in the precipitation or maintenance of the disease process cannot be excluded (BLIZZARD, 1967). Whereas disorders such as pernicious anemia and myasthenia gravis, considered to be organ-specific autoimmune diseases, have been observed with the autoimmune polyendocrinopathy, it is not known whether the so-called collagen diseases with organ-nonspecific antibodies to components of the cell nucleus, e. g. lupus erythematosus, occur more frequently than just by chance (BECKER, 1965). Besides hypothyroidism and Hashimoto's thyroiditis, hyperthyroidism is the only hyperfunctional syndrome which frequently occurs concomitantly with primary adrenal atrophy. This may be due to the immunological processes involved in thyrotoxicosis and the possible transition of hyper- into hypothyroidism. Immunological processes today also occupy a prominent position in the pathogenesis of diabetes mellitus (see p. 762), and antibodies to the thyroid gland and the gastric mucosa are found more fre-

quently in diabetics than in non-diabetics (MOORE, 1964). In addition to gastritis, latent or manifest malabsorption also occurs and may have an immunological basis (SIURALA, 1968). The occurence of endocrine adenomatosis and endocrine autoimmune-polyadenopathy, which has recently been observed in one family, remains unexplained (MERSHON, 1966). The role of the giant-cell granulomas in individual endocrine glands described in the earlier literature is not yet known.

3. Clinical Features

Adrenal insufficiency dominates the clinical picture. It usually, but by no means always, becomes manifest first, causes most of the complaints and endangers the patient most. Pigmentations are present and sometimes vitiligo; the latter may have a common autoimmune pathogenesis (MCGREGOR, 1967). They are, however, not so striking as in the usual Addison's disease, especially if hypothyroidism with pallor and myxedematous skin develops. The lack of animation and the bloated expressionless features contrast to the sharply chiselled face of the Addisonian patient, and may give a clinical indication of associated hypothyroidism, If the gonads are also involved and there is absence of axillary and pubic hair and atrophic testes in the male or amenorrhea in the female, differentiation from hypopituitarism is difficult and only the pigmentation instead of the pallor suggests a primary glandular disorder. The diagnosis must be confirmed by laboratory tests when pituitary insufficiency is suspected; if the suspicion is correct an increase instead of an absence of gonadotropins will be revealed. Negative ACTH and TSH tests confirm the diagnosis. Occasionally, resorption is also impaired (SIURALA, 1968).

It is difficult to control the diabetes in spite of cortisone substitution, and the long-term prognosis is not favorable even with correct replacement therapy.

It has long been observed that hyperthyroidism and hypothyroidism (GASTINEAU, 1964, 1963) and diabetes mellitus (BEAVEN, 1959) occur frequently in Addison's disease. It is therefore worthwhile to examine thyroid function and to estimate the calcium and blood sugar in Addison's disease, especially in those cases with no history of tuberculosis. Wherever there are facilities, antibodies to the adrenals, the thyroid gland, the parathyroids and the gastric mucosa should be investigated. This enables the concomitant disorder to be detected before it becomes manifest. On the other hand, it is not worthwhile to undertake the systematic tests for concomitant endocrine disorders in hypothyroidism (BLIZZARD, 1967).

The treatment is no different from that for ADDISON's disease, hypothyroidism, and diabetes mellitus.

C. Syndrome of Diabetes Mellitus, Diabetes Insipidus, and Optic Atrophy

This rare syndrome has been observed in eight members of five families and is probably transmitted by an autosomal recessive mode of inheritance (BRETZ, 1970).

References

Endocrine Adenomatoses

BALLARD, H.S., RAME, B., HARTSOCK, R.J.: Familial multiple endocrine adenoma-peptic ulcer complex. Medicine (Baltimore) **43**, 481 (1964).

BECKER, V., SCHNEIDER, H.H.: Das Wermer-Syndrom: hereditäre endokrine Polyadenomatose. Dtsch. med. Wschr. **93**, 935 (1968).

BERDJIS-CHAMSI, C.: Pluriglandular syndrome. I. Multiple endocrine adenoma in irradiated rats. Oncologia (Basel) **13**, 441 (1960).

CUNLIFFE, W.J., HUDGSON, P., FULTHORPE, J.J., BLACK, M.M., HALL, R., JOHNSTON, I.D.A., SCHUSTER, S.: A calcitonin-secreting medullary thyroid carcinoma associated with mucosal neuromas, marfanoid features, myopathy and pigmentation. Amer. J. Med. **48**, 120 (1970).

DONAHOWER, G.F., SCHUMACHER, O.P., HAZARD, J.B.: Medullary carcinoma of the thyroid—a cause of Cushing's syndrome: Report of two cases. J. clin. Endocr. **28**, 1199 (1968).

ELLENBERG, A.L., GOLDMANN, L., GORDON, G.S., LINDSAY, S.: Thyroid carcinoma in patients with hyperparathyroidism. Surgery **51**, 708 (1962).

ERDHEIM, J.: Zur normalen und pathologischen Histologie der glandula thyreoidea, Parathyreoidea und Hypophysis. Beitr. Path. Anat. **33**, 158 (1903).

FEYRTER, F.: Über die peripheren endokrinen (parakrinen) Drüsen des Menschen. Wien-Düsseldorf: Maudrich 1953.

FISCHER, J.A., OLDHAM, S.B., SIZEMORE, G.W., ARNAUD, C.D.: Calcitonin-stimulation of parathyroid hormone secretion in vitro. Hormone Metab. Res. **3**, 223 (1971).

JACKSON, CH.E., BOONSTRA, CH.E.: The relationship of hereditary hyperparathyroidism to endocrine adenomatosis. Amer. J. Med. **43**, 727 (1967).

JOHNSON, G.J., SUMMERSKILL, W.H., ANDERSON, V.E.: Clinical and genetic investigation of a large kindred with multiple endocrine adenomatosis. New Engl. J. Med. **277**, 1379 (1967).

KERR, G.D., SMITH, R.: Hypercalcaemia and gastric hypersecretion in the familial endocrine-adenoma syndrome. Lancet **1967 I**, 1074.

KEYNES, W.M., TILL, A.S.: Medullary carcinoma of the thyroid gland. Quart. J. Med. **60**, 443 (1971).

LAMBERG, B.A., RIPATTI, J., GORDON, A., JUUSTILA, H., SIVULA, A., BJÖRKESTEN, G.: Chromophob pituitary adenoma with acromegaly and TSH-induced hyperthyroidism associated with parathyroid adenoma: acromegaly and parathyroid adenoma. Acta endocr. (Kbh.) **60**, 157 (1969).

MCGREGOR, B.C., KATZ, H.I., DOE, R.P.: Vitiligo and multiple glandular insufficiencies. J. Amer. med. Ass. **219**, 724 (1972).

MANNING, P. C., JR., MOLNAR, G. D., BLACK, B. M., PRIESTLEY, J. T., WOOLNER, L. B.: Phaeochromocytoma, hyperparathyroidism and thyroid carcinoma occurring coincidentally. New Engl. J. Med. **268**, 68 (1963).

MELVIN, K. E. W., TASHJIAN, A. H., JR., MILLER, H. H.: Studies in familial (medullary) thyroid carcinoma. Recent Progr. Hormone Res. **28**, 399 (1972).

MOLDAWER, M.: Multiple endocrine tumors and Zollinger-Ellison-Syndrome in families: One or two syndromes? Report of two new families. Metabolism **11**, 152 (1962).

PEARSE, A. G. E.: Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial cells and calcitonin. Proc. roy. Soc. B **170**, 71 (1968).

— EWEN, S. W. B., POLAK, J. M.: The genesis of apud-amyloid in endocrine polypeptide tumors: Histochemical distinction from immunamyloid. Virchows Arch. Abt. B **10**, 93 (1972).

— POLAK, J. M.: Neural crest origin of endocrine polypeptide (APUD) cells of gastrointestinal tract and pancreas. Gut **12**, 783 (1971).

RENWICK, S. W., ROYLE, J. P.: Pluriglandular syndrome with hyperparathyroidism and Zollinger-Ellison-Syndrome. Proc. roy. Soc. Med. **61**, 87 (1968).

ROSSIER, P. H., DRESSLER, M.: Familiäre Erkrankung innersekretorischer Drüsen kombiniert mit Ulcuskrankheit. Schweiz. med. Wschr. **69**, 985 (1939).

RUTISHAUSER, E.: Hypophyseo-parathyreotisches Syndrom. Zbl. allg. Path. path. Anat. **80**, 193 (1943).

SAROSI, G., DOE, R. P.: Familial occurrence of parathyroid adenomas, pheochromocytoma and medullary carcinoma of the thyroid with amyloid struma (Sipple's syndrome). Ann. intern. Med. **68**, 1305 (1968).

SCHIMKE, R. N., HARTMANN, W. H., PROUT, TH. E., RIMOIN, D. L.: Syndrome of bilateral pheochromocytoma, medullary thyroid carcinoma and multiple neuromas. A possible regulatory defect in the differentiation of chromaffin tissue. New Engl. J. Med. **279**, 1 (1968).

SCHMID, J., LABHART, A., ROSSIER, P. H.: Relationship of multiple endocrine adenoma to the syndrome of ulcerogenic isletcell-adenomas (Zollinger-Ellison). Amer. J. Med. **31**, 343 (1961).

SIPPLE, J. H.: The association of pheochromocytoma with carcinoma of the thyroid gland. Amer. J. Med. **31**, 163 (1961).

STEINER, H., HEITZ, PH.: Die pluriglandulären endokrinen Regulationsstörungen in endokrinen Organen. Spezielle pathologische Anatomie. Ed. by DOERR, SEIFERT und UEHLINGER. Berlin-Heidelberg-New York: Springer (im Druck).

STEINER, H. L., GOODMAN, A. D., POWERS, S. R.: Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrinopathia type 2. Medicine (Baltimore) **47**, 371 (1968).

STUDNITZ, W., LJUNGBERG, O.: Familiär auftretendes adrenalinproduzierendes Phäochromozytom in Kombination mit medullärem Thyreoidea-Carcinom. Klin. Wschr. **48**, 144 (1970).

SWINTON, N. W., JR., CLERKIN, E. PL., FLINT, L. D.: Hypercalcemia and familial pheochromocytoma. Ann. intern. Med. **76**, 455 (1972).

UNDERDAHL, L. O., WOOLNER, L. B., BLACK, B. R.: Multiple endocrine adenomas. Report of 8 cases in which parathyroids, pituitary and pancreatic islets were involved. J. clin. Endocr. **13**, 20 (1953).

VANCE, J. E., STOLL, R. W., KITABCHI, A. E., WILLIAMS, R. H., WOOD, F. C., JR.: Nesidioblastosis in familial endocrine adenomatosis. J. Amer. med. Ass. **207**, 1679 (1969).

WEICHERT, R. F.: The neural ectodermal origin of the peptide-secreting endocrine glands. A unifying concept for the etiology of multiple endocrine adenomatosis and the inappropriate secretion of peptide hormones by non-endocrine tumors. Amer. J. Med. **49**, 232 (1970).

WERMER, P.: Genetic aspects of adenomatosis of endocrine glands. Amer. J. Med. **16**, 363 (1954).

— Endocrine adenomatosis and peptic ulcer in a large kindred. Amer. J. Med. **35**, 305 (1963).

WERNLY, M., BERDJIS-CHAMSI, C.: Les parathyréoides humaines. Basel: Schwabe 1956.

WILLIAMS, E. D., CELESTIN, L. R.: The association of bronchial carcinoid and pluriglandular adenomatosis. Thorax **17**, 120 (1962).

Autoimmune-Polyendocrinopathy

ANDERSON, J. R., BUCHANAL, W. W., GOUDIE, R. B.: Autoimmunity. Clinical and experimental. Springfield: Thomas 1967.

BAMATTER, F., KOEGEL, R., HALLER, J. DE, MULLER, A. F.: Maladie d'Addison familiale: insuffisance corticosurrénale chez 4 frères et plusieurs cas probables dans deux générations de la même famille. Helv. paediat. Acta **21**, 109 (1966).

BEAVEN, D. W., NELSON, D. H., RENOLD, A. E., THORN, G. W.: Diabetes mellitus and Addison's disease. Report on 8 patients and a review of 55 cases in literature. New Engl. J. Med. **261**, 443 (1959).

BECKER, K., FERGUSON, R. H., MCCONAKEY, W. M.: The connective-tissue diseases and symptoms associated with Hashimoto's thyroiditis. New Engl. J. Med. **268**, 277 (1963).

BLIZZARD, R. M., KYLE, M.: Studies of the adrenal antigen and antibodies in Addison's disease. J. clin. Invest. **42**, 1653 (1963).

— SHEE, D., DAVIS, W.: The incidence of adrenal and other antibodies in the sera of patients with idiopathic adrenal insufficiency (Addison's disease). Clin. exp. Immunol. **2**, 19 (1967).

— TOMASI, P. B., CHRISTY, N. P.: Autoantibodies against thyroid and adrenal tissue in a patient with multiple primary endocrine deficiencies. J. clin. Endocr. **23**, 1179 (1963).

BRETZ, G. W., BAGHDARSARIAN, A., GRABER, J. D., ZACHERLE, B. J., NORUM, R. A., BLIZZARD, R. M.: Coexistence of diabetes mellitus and insipidus and optic atropy in two male siblings. Amer. J. Med. **48**, 398 (1970).

BRONSKY, B., MELTZER, J. L., WALDENSTEIN, S. S.: Idiopathic juvenile myxoedema and myasthenia gravis. Amer. J. Med. **43**, 956 (1967).

CARPENTER, CH. C. J., SOLOMON, N., SILVERBERG, S. G., BLEDSOE, T., NORTHCUTT, R. C., KLINENBERG, J. R., BENNETT, I. L., JR., HARVEY, A. M.: Schmidt's syndrome (thyroid and adrenal insufficiency): A review of the literature and a report of 15 new cases including ten instances of co-existent diabetes mellitus. Medicine (Baltimore) **43**, 153 (1964).

CHRISTY, N. P., HOLUB, D. A., TOMASI, T. B.: Primary ovarian, thyroidal and adrenocortical deficiencies simulating pituitary insufficiency, associated with diabetes mellitus. J. clin. Endocr. **22**, 155 (1962).

CLAUDE, H., GOUGEROT, H.: Sur l'insuffisance simultanée de plusieurs glandes à secrétion interne (insuffisance pluriglandulaire). J. Physiol. Path. gén. **10**, 468, 505 (1908).

EGLOFF, B., FISCHBACHER, W., GOUMOENS, E. VON: Lymphomatöse Hypophysitis mit Hypophyseninsuffizienz. Schweiz. med. Wschr. **99**, 1499 (1969).

EVANS, A. W. H., WOODROW, J. C., MCDOUGALL, C. D. M., CHEW, A. R., EVANS, R. W.: Antibodies in the families of thyreotoxic patients. Lancet **1967 I**, 636.

FALTA, W.: Späteunuchoidismus und multiple Blutdrüsensklerose. II. Multiple Blutdrüsensklerose. Berl. klin. Wschr. **49**, 1477 (1912).

FEDERLIN, K.: Autoimmunophänomene bei Erkrankungen endokriner Drüsen. Klin. Wschr. **7**, 337 (1969).

GASTINEAU, C. F., ARNOLD, J. W.: Clinics on endocrine and metabolic diseases. 12. Thyroid disorders in Addison's disease. 1. Myxoedema and goiter. Proc. Mayo Clin. **38**, 323 (1963).

— MYERS, W. R., ARNOLD, J. W., MCCONAHEY, W. M.: Thyroid disorders in Addison's disease. 2. Grave's disease. Proc. Mayo Clin. **39**, 939 (1964).

GOLONKA, J. E., GOODMAN, A. D.: Coexistence of primary ovarian insufficiency, primary adrenocortical insufficiency and idiopathic hypoparathyroidism. J. clin. Endocr. **28**, 79 (1968).

GOUDIE, R. B., PINKTERTON, P. H.: Anterior hypophysitis and Hashimoto's disease in a young woman. J. Path. Bact. **83**, 584 (1962).

GUISAN, M., RIBAS-MUNDO, M., LABHART, A.: Syndrome de Schmidt (maladie d'Addison et hypothyroidie idiopathique) et diabète sucré. Schweiz. med. Wschr. **99**, 1341 (1969).

HONG, W., MIGEON, C. J., PARROTT, R. H.: A possible autoimmune basis for Addison's disease in 3 siblings, one with idiopathic hypoparathyroidism, pernicious anemia and superficial moniliasis. New Engl. J. Med. **269**, 658 (1963).

HUME, R., ROBERTS, G. H.: Hypophysitis and hypopituitarism: report of a case. Brit. med. J. **1967 I**, 548.

IRVINE, W. J.: A clinical and immunological study of adrenal insufficiency. J. Endocr. **26**, XXXII (1963).

— CHAN, M. N. W., SCARTH, L., KOLB, F. O., HARTOG, M., BAYLISS, R. I. S., DRURY, M. I.: Immunological aspects of premature ovarian failure associated with idiopathic Addison's disease. Lancet **1968 II**, 883.

— CLARKE, B. F., SCARTH, L., CULLEN, D. R., DUNCAN, L. J. P.: Thyroid and gastric autoimmunity in patients with diabetes mellitus. Lancet **1970 I**, 163.

– STEWART, A. G., SKARTH, L.: A clinical and immunological study of adrenocortical insufficiency (Addison's disease). Clin. exp. Immunol. **2**, 31 (1967).

KRA, S. J., BARILE, A. W.: Addison's disease and Addisonian anemia. Arch. intern. Med. **114**, 258 (1964).

KUNIN, A. S., MACKAY, B. R., BURNS, S. L., HALBERSTAM, M. J.: The syndrome of hypoparathyroidism and adrenocortical insufficiency, a possible sequal of hepatitis. Amer. J. Med. **34**, 856 (1963).

Leading article: Autoimmunity in idiopathic Addison's disease. Lancet **1967 I**, 1040.

LECOMPTE, PH. M., STEINKE, J., SOELDNER, J., RENOLD, A. E.: Changes in the islets of Langerhans in cows injected with heterologous and homologous insulin. Diabetes **15**, 586 (1966).

LEVINE, S.: Allergic adenohypophysitis: New experimental disease of the pituitary gland. Science **158**, 1190 (1967).

MEECHAM, J., JONES, E. W.: Addison's disease and Addisonian anaemia. Lancet **1967 I**, 535.

MERSHON, J. C., DIETRICH, J. G.: Hereditary Addison's disease and multiple endocrine adenomatosis in a kindred. Ann. intern. Med. **65**, 252 (1966).

MICHEL, H.: Endokrinologie und Autoaggression. Internist (Berl.) **6**, 134 (1965).

MOORE, J. M., NEILSON, J. MC E.: Antibodies to gastric mucosa and thyroid in diabetes mellitus. Lancet **1964 II**, 645.

MORSE, W. J., COCHRANE, W. A., LANDRISAN, P. L.: Familial hypoparathyroidism with pernicious anemia, steatorrhea and adrenocortical insufficiency. A variant of mucoviscidosis. New Engl. J. Med. **264**, 1921 (1961).

NERUP, J., SOBORG, M., HALBERG, P., BROCHNER-MORTENSEN, K.: Organ specific antibodies in Addison's disease. Acta med. scand. Suppl., 445, ad **179**, 383 (1966).

RICKARDS, A. G., BARRETT, G. M.: Non tuberculous Addison's disease and its relationship to giant cell granuloma and multiple glandular disease. Quart. J. Med. **23**, 403 (1954).

SCHMIDT, M. B.: Eine biglanduläre Erkrankung (Nebennieren und Schilddrüse) bei Morbus Addisonii. Verh. dtsch. Path. Ges. **21**, 21 (1926).

SIMPSON, J. A.: Immunological disturbances, myasthenia gravis with a report of Hashimoto's disease developping after Thymectomy. J. Neurol. Neurosurg. Psychiat. **27**, 495 (1964).

SINGER, W., SAHAY, B. M.: Myasthenia gravis, Hashimoto's thyroiditis and pernicious anaemia. Brit. med. J. **1966 I**, 904.

SIURALA, M., VARIS, K., LAMBERG, B. A.: Intestinal absorption and autoimmunity in endocrine disorders. Acta med. scand. **184**, 53 (1968).

SOLOMON, N., CARPENTER, CH. C. J., BENNETT, J. L., JR., HARVEY, A. M.: Schmidt's syndrome (thyroid and adrenal insufficiency) and co-existant diabetes mellitus. Diabetes **14**, 300 (1965).

TURKINGTON, R. W., LEBOVITZ, H. E.: Extra-adrenal endocrine deficiencies in Addison's disease. Amer. J. Med. **43**, 499 (1967).

TZAGOURNIS, M., HAMROI, G. J.: The prevalence of Diabetes mellitus in patients with Addison's disease: Measurements of serum insulin levels. Metabolism **16**, 213 (1967).

VALLOTTON, M. B., FORBES, A.: Autoimmunity in gonadal dysgenesis and Klinefelter's syndrome. Lancet **1967 I**, 652.

WITEBSKIY, E., MILGROM, F.: Immunological studies on adrenal glands. II. Immunization with adrenals of the same species. Immunology **5**, 67 (1962).

WUEPPER, K. D., FUDENBERG, H. H.: Moniliasis, "autoimmune" polyendocrinopathy, and immunologic family study. Clin. exp. Immunol. **2**, 71 (1967).

XIX. Growth and Development

A. PRADER

A. General Concepts of Growth and Development

Growth is due to an increase in the number and size of the body cells and to the development of cartilagenous and osseous substances. Anabolism exceeds catabolism in the continuous process of transformation of protoplasm and bone tissue in the growing organism. In other words, growth is an *anabolic process*. This process is characterized clinically by an increase in height and weight, and metabolically in particular by retention (positive balance) of water, nitrogen and electrolytes.

Development, i.e. the morphological differentiation and functional specialization of certain parts of the organism, progresses parallel to growth.

The final size of an adult depends both on the rate of growth and on the duration of growth. Longitudinal growth, however, is only possible as long as the epiphyses of the bones are not closed. Consideration of the *maturation of the bones* is thus of great significance for an understanding of growth. Bone maturation does not imply longitudinal growth of bones but rather the degree of ossification which is radiologically detectable (p. 1026).

Almost all endocrine disorders beginning in the first 2 years of life have a decisive influence on growth and development. The deviation of growth and development from normal often indicates not only the type of disorder but also the time of onset. Precise knowledge of normal and pathologic growth and development is therefore indispensable in pediatric endocrinology.

1. General Factors of Growth

As far is known, conditions necessary for normal growth can be summarized (Fig. 1) as follows:

1. *Building material* obtained from the nourishment must be completely adequate in quality and quantity. The more marked growth in the upper social classes and the acceleration of growth observed over the past 100–150 years are probably due to better nutrition. *Psychological factors* can also affect the intake of food and thus growth and development.

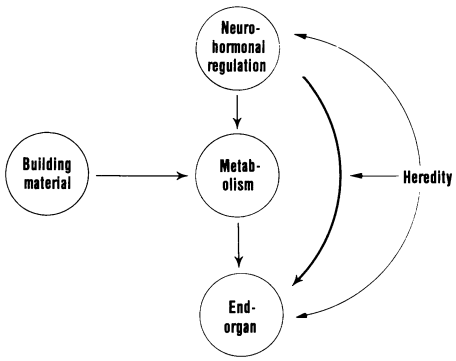


Fig. 1. General growth factors

2. *Neurohormonal regulation.* The hypothalamo-hypophyseal system is responsible for the integration of all the growth-regulating influences. The influence of hormones is discussed in detail on p. 1016. Cerebral and hormonal disorders can result in severe growth disturbances.

3. *Metabolism.* The building material available must be properly absorbed, transported and assimilated. The intestinal, cardiac, renal and other forms of dwarfism are due to impairments of these metabolic processes.

4. *The end organ* (protoplasm, bones) must posses a normal *growth potential.* Chondrodystrophy is an example of reduced growth potential of the long bones.

5. *Genetic factors* can influence growth via points 2–4.

2. Body Growth and Growth of the Endocrine Glands

Growth and development of the body show many features related more or less closely to the weight curve of the endocrine glands and the excretion of hormones in the urine. The growth curves of the whole organism, the individual organic systems and endocrine glands

are not at all similar. Broadly speaking, the following types of growth can be recognized.

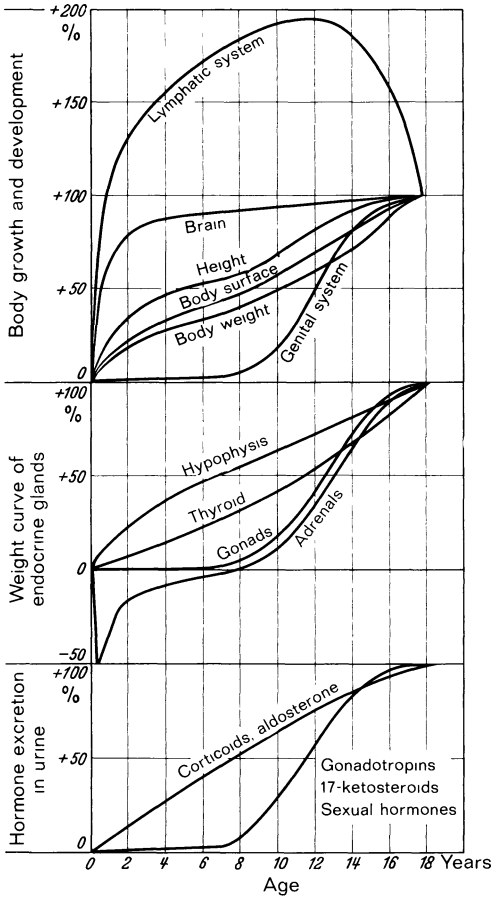


Fig. 2. Graph showing body growth, growth of the endocrine glands and increase in hormone excretion from birth to adulthood (as percentages of the postnatal increase in size) (PRADER, 1956)

a) General Growth

Increases in height and weight, in body surface, musculature, skeleton and other organs all demonstrate a similar type of growth at first glance. On closer analysis, however, four different phases can be recognized. To begin with, the body grows extremely quickly. This is followed by a slower but quite constant period of growth extending to puberty. In this phase the growth curve is roughly parallel to the weight curves of the hypophysis and the thyroid gland. Puberty is accompanied by a new spurt of growth which is obviously related to the sudden increase in weight of the gonads and adrenals and the production of sexual hormones. The pubertal spurt in growth gradually subsides and is followed by an arrest of growth.

b) Neural Growth

There are no parallels in growth of the endocrine glands and in the excretion of hormones corresponding to the disproportionately rapid increase in the circumference of the head and the weight of the brain during the first 3 years of life, followed by the insignificant growth in subsequent years. This type of growth is probably largely independent of hormonal factors.

c) Lymphatic Growth

Growth of the lymphatic system (measured from the weight of the thymus) is characteristically more intense and more prolonged. A strong involutional process begins at puberty and a temporal connection with the accelerated growth of the gonads and adrenals cannot be mistaken.

d) Genital Growth

The genital system grows very little before puberty and then completes its entire phase of growth within a few years. The weights of the gonads and adrenals and the excretion of gonadotropin and sexual hormones follow a similar course and show that genital development is dependent on the hypophysis, gonads and adrenals.

The growth curve of the adrenals is the most striking of all. Apart from minimal fetal hyperplasia and postnatal involution, which are still not understood (p. 286), before puberty the curve corresponds to the steady increase in the excretion of corticoids and aldosterone and during puberty to the sudden increase in the weight of the gonads and excretion of gonadotropins and sexual hormones. The shape of this curve reflects the uniform increases in the production of gluco- and mineralocorticoids on one hand, and the rapidly rising production of adrenocortical androgens (adrenarche, p. 1039) beginning in puberty on the other.

B. Hormonal Influence on Growth and Bone Development

Hypophyseal growth hormone (STH), thyroid hormones and probably insulin have a decisive effect on growth as a whole. In contrast to this prolonged action, gonadal hormones and probably certain adrenocortical hormones promote growth and bone development only during puberty. A growth-promoting action was attributed to the thymus earlier, but this has never been confirmed.

True *growth hormones* are only those with protein-anabolic, i.e. nitrogen-retaining actions such as STH, insulin, and testosterone. Other hormones, with no anabolic action, such as thyroxine, are also essential for normal growth but cannot be termed true growth hormones.

Table 1. Influence of hormones on growth and bone development

	Growth	Bone development
Hypophyseal growth hormone (STH)	+ + +	+
Thyroid hormones	+	+ +
Insulin	?+	?+
Glucocorticoids	−	−
Androgens*	+	+ +
Ovarian estrogens*	0	?+

+ promoting action, 0 no effect, − inhibitory action, * only during adolescence.

Table 2. Growth and bone development in over- and underproduction of hormones

	Growth	Bone development
Hyperfunction		
STH (hypophyseal gigantism)	+ + +	0−
Thyroid hormones (hyperthyroidism)	+	+
Insulin (islet-cell adenoma)	+	?
Adrenocortical glucocorticoids (Cushing's syndrome)	− −	− −
Adrenocortical androgens (adrenogenital syndrome)	+ +	+ + +
Testicular androgens (Leydig cell tumor)	+ +	+ + +
Ovarian estrogens (Granulosa cell tumor)	+	+ +
Hypofunction		
STH (hypophyseal dwarfism)	− − −	− −
Thyroid hormones (hypothyroidism)	− −	− − −
Insulin (diabetes mellitus)	−	−
Adrenocortical steroids (Addison's disease)	? 0	? 0
Testicular androgens (hypogonadism)*	0	−
Ovarian estrogens (hypogonadism)*	0	−

+ accelerated, 0 uninfluenced, − retarded, * only during adolescence.

1. Survey

Tables 1 and 2 give a schematic survey of the present-day interpretation of the action of various hormones on growth and bone development in normal and pathologic conditions.

2. Hypophyseal Growth Hormone (STH, GH)

In pituitary gigantism (p. 107), growth is greatly accelerated whereas bone development is normal or delayed. In pituitary dwarfism (p. 98), due to an isolated STH deficiency, growth is greatly retarded and bone development only slightly retarded.

Experimentally, STH has anabolic and growth-promoting actions (p. 88). In rats, hypophyseal gigantism can be induced by injection of STH, and hypophyseal dwarfism by hypophysectomy. In spite of its short half-life (p. 84) and the irregular fluctuation of its plasma concentration, the growth-promoting effect of STH is constant, exerted not directly but indirectly via the sulfation (p. 88) and thymidine factors to enhance protein synthesis. This action can easily be measured by means of nitrogen retention. Administration of exogenous STH produces much better nitrogen retention and growth in patients with endogenous STH deficiency than in patients with normal endogenous STH production (p. 101). The optimum effect, however, is only attained in the presence of thyroxine and insulin.

STH is demonstrable in the hypophysis and plasma of the human fetus from the 4th fetal month. Since STH cannot pass through the placenta, there is no exchange between maternal and fetal STH. STH concentration in the plasma of the newborn is considerably higher than in the mother. It therefore appears probable that prenatal and neonatal growth, characterized predominantly by an increase in the number of cells, is related to the increased plasma concentration of STH. In fact, promotion of cellular multiplication is the main function of STH, and it also promotes cell growth but to a lesser degree (Cheek, 1970). The fact that anencephalic babies are of more or less normal size at birth led to the interpretation in the past that STH was not essential for fetal growth. Further support for this assumption was found in the fact that prenatal growth and growth in the first months of life were normal in pituitary dwarfism. However, this interpretation does not take into account the fact that most cases of STH deficiency were due to cerebral injury at delivery and that the effects of this could only arise postnatally. It now appears more likely that in prenatal STH deficiency body

size compared to weight is slightly impaired even at birth. It can be assumed from this that prenatal and neonatal growth are at least partly dependent on STH.

Serum phosphorus and alkaline phosphatases are higher in the growing child than in the adult. The concentration of phosphorus can be considered an index of STH production, and alkaline phosphatase an index of osteoblastic activity. In overproduction of STH (pituitary gigantism, acromegaly) the serum phosphorus is elevated but is normalized by the administration of androgenic or estrogenic hormones. Serum phosphorus is diminished in STH deficiency and rises on administration of human growth hormone. In rats, treatment with STH leads to a rise of phosphorus and alkaline phosphatase in the serum whereas hypophysectomy results in a fall.

3. Thyroid Hormones

One of the chief results of infantile hypothyroidism (p. 165) and thyroidectomy in the young animal is inhibition of growth and bone development. As a rule, bone age is more retarded than growth. In contrast to this, growth and bone development are usually somewhat accelerated in infantile hyperthyroidism. These disorders can easily be imitated experimentally in animals.

Thyroid hormones are formed by the fetal thyroid gland. Because of this, bone maturation and brain development are found to be retarded even at birth in cases of congenital athyroidism. Only small amounts of thyroid hormones pass through the placenta, and maternal thyroid hormones are therefore unable to compensate fully for the absence of the fetal thyroid gland.

Thyroid hormones are responsible for normal postnatal growth and, even more, for normal bone maturation. Physiological doses produce this effect only in athyroidism and hypothyroidism. The presence of STH is essential. In the presence of STH deficiency thyroid hormones can only correct the delay in bone maturation and not the delay in growth. Higher doses accelerate bone maturation considerably and only slightly affect growth in normal subjects.

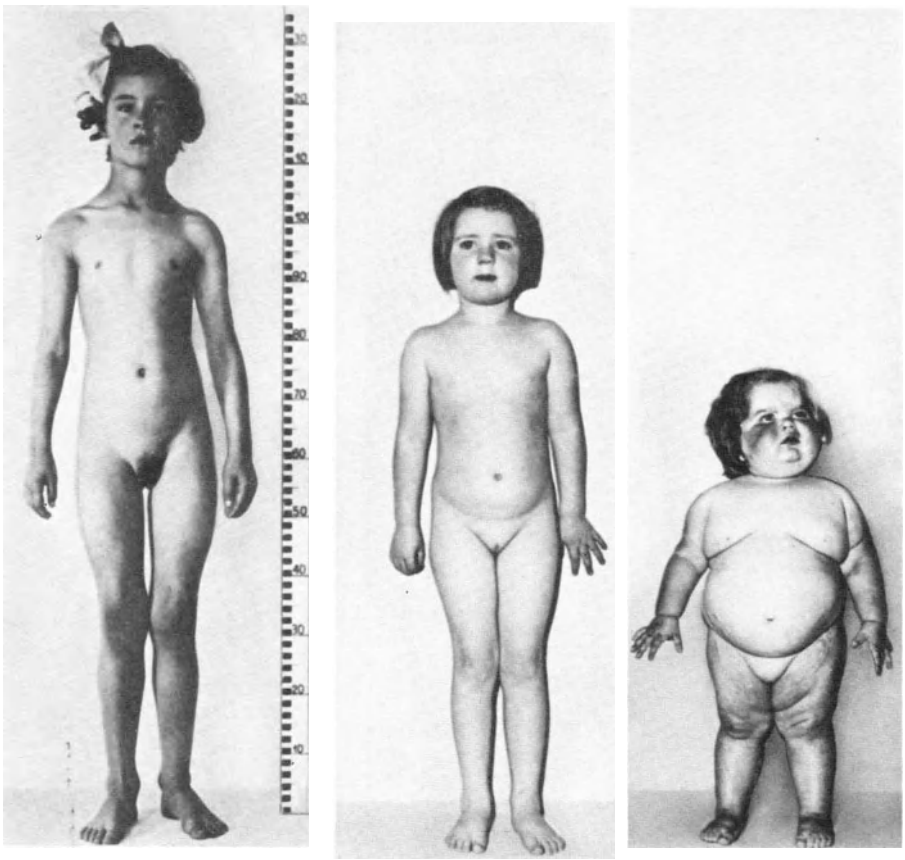


Fig. 3. Influence of adrenocortical hyperfunction on growth. The three girls are all the same age (4–4.5 years). The increased production of anabolic adrenocortical androgens in the adrenogenital syndrome (left) promotes growth. The increased production of catabolic glucocorticoids in Cushing's syndrome inhibits growth (right) (PRADER, 1956)

In hypothyroidism, growth retardation is probably not only due to deficiency of thyroid hormones but also to a secondary STH deficiency. The rise in STH is found to be inadequate after hypoglycemia induced by insulin. This defect disappears on treatment with thyroid hormone. Besides the change in the basophil cells in the hypophysis (p. 80) a decrease in the number of eosinophil cells, which presumably form STH, following thyroidectomy is consistent with this viewpoint.

4. Insulin and Glucagon

Normal protein anabolism in the organism can only occur in the presence of adequate amounts of insulin. It is therefore assumed that *insulin* is essential for the anabolic and growth-promoting action of STH. This interpretation is supported by the facts that growth is retarded in badly treated juvenile diabetics and that growth is rather accelerated in patients with islet-cell tumors. In over-weight babies of diabetic mothers and in infantile obesity, mild gigantism is a characteristic feature. Provocation methods in both instances cause a pronounced rise in insulin, whereas the rise in STH is reduced. Conversely, after the administration of large amounts of glucose to patients with STH deficiency, the insulin rise is diminished and can be normalized by treatment with human STH.

Very little is known about the action of glucagon on growth. Nevertheless, the STH concentration in the blood can be stimulated by glucagon in normal conditions.

5. Adrenocortical Hormones

Over-production of the anabolic-acting androgenic adrenocortical hormones results in the adrenogenital syndrome (p. 358). Growth and bone development are greatly accelerated in these patients (Fig. 4). Since bone development is accelerated more than growth, epiphyseal fusion and arrest of growth occur before normal adult height is attained (Fig. 29). The gigantism in childhood is thus replaced by dwarfism in adulthood.

Over-production of the catabolic-acting glucocorticoids is present in Cushing's syndrome (p. 340). In the pure Cushing's syndrome during childhood, growth and bone development are greatly inhibited (Fig. 3). Analogously, long-term cortisone treatment in children leads to definite inhibition of growth which can usually be recompensated after treatment has been terminated. The effect may be due in part to a secondary STH deficiency. In fact, the STH level in the plasma is reduced in Cushing's

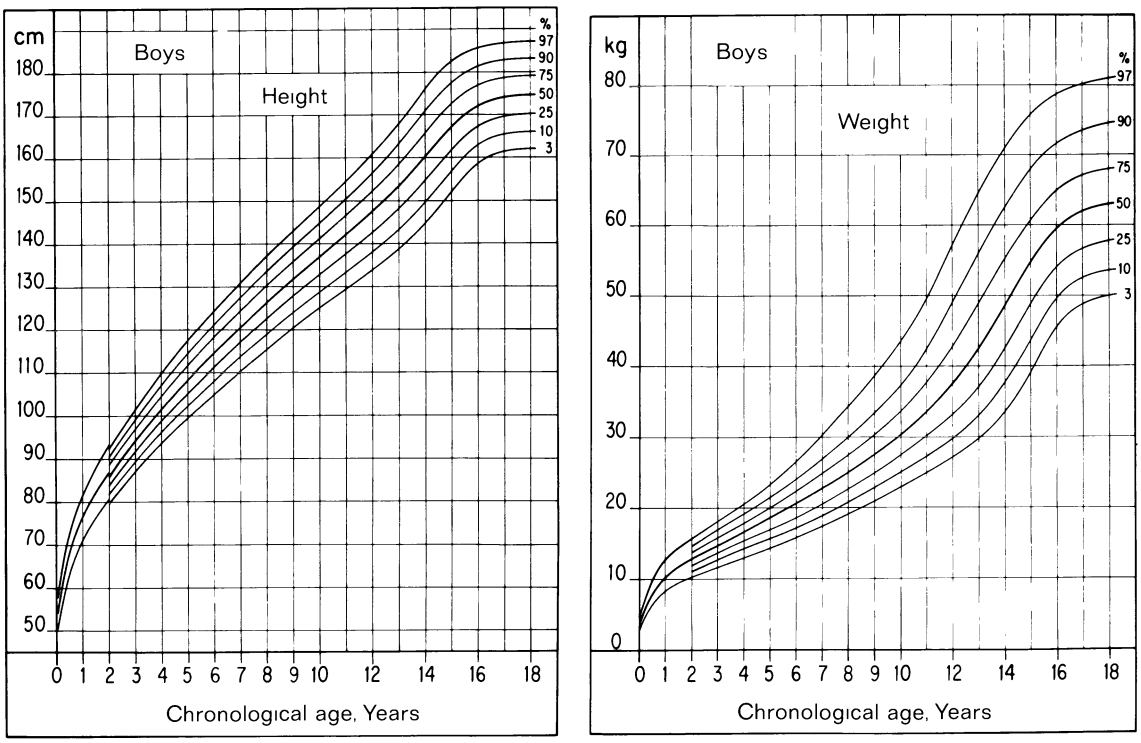


Fig. 4. Height and weight of boys (TANNER, 1966). Percentile 50 represents median values. Percentile 10 (or 90) signifies that 10% (or 90%) of all subjects of the same age are shorter or lighter and 90% (or 10%) are taller or heavier

syndrome and during treatment with high doses of cortisone, secretion and the peripheral anabolic action of STH being more severely impaired than its production. During therapeutic use of glucocorticoids it must be borne in mind that cortisone and cortisol inhibit growth to a lesser extent than prednisone and prednisolone and that equivalent doses of the other synthetic glucocorticoids have an even greater growth-inhibiting action. The latter compounds should therefore never be used during childhood. The growth-inhibiting action is even slighter if cortisone is given every other day or if ACTH is used instead.

In *chronic adrenocortical insufficiency* such as is seen in Addison's disease and in adrenalectomized rats, growth is only inhibited if inadequate amounts of salt and cortexone (desoxycorticosterone) are given. Bone development is almost normal.

In summary, growth and bone development are promoted by androgenic adrenocortical hormones and inhibited by glucocorticoids (Fig. 3). Growth and bone development remain normal in the absence of both types of hormones. The influence of adrenocortical hormones on normal growth in puberty is discussed on p. 1021.

6. Gonadal Hormones

Up to shortly before puberty, the gonads produce only very small amounts of hormones. During normal puberty as well as in premature puberty and in the absence of puberty, growth and bone development show characteristic features associated with the endocrine function of the gonads.

a) Testicular Androgens

Over-production of androgens by the testes occurs only with Leydig-cell tumors (p. 1051). The clinical picture is similar to that of precocious pseudopuberty (p. 1045). As with the overproduction of adrenocortical hormones, growth and bone development are accelerated, especially the latter. The same is applicable to true precocious puberty (p. 1045), where the testes produce androgens prematurely but in the normal manner. In this condition too, the result of the accelerated bone development is that growth is arrested even before normal adult height has been reached.

Failure of the testes to produce androgens, pre-puberal castration and eunuchoidism (p. 457) result in partial inhibition of bone development while growth is not suppressed. The spurt of growth during puberty fails to occur but delay testes in the male or amenorrhea in the female. duration of growth. The result is a height somewhat above the normal and strikingly long extremities (eunuchoid gigantism, p. 458).

Testosterone, the androgenic hormone secreted by the testes, has a very strong anabolic action (p. 453). Like the endogenous androgen production, testosterone used therapeutically during the period of growth results in acceleration of growth and bone development, the latter being the more markedly affected. In the presence of an STH deficiency, the anabolic action of testosterone promoting growth and the androgenic effect are reduced (p. 102). Conversely, testosterone enhances STH secretion: there is a greater rise is plasma STH after provocation in a child receiving testosterone, and the increase is greater in adults than in children.

b) Ovarian Estrogens

Primary *overproduction of estrogens* during childhood due to granulosa-cell tumors (p. 1050) leads to the picture of pseudosexual precocity (p. 1045). Growth and bone maturation are usually accelerated as in true precocious puberty and in the adrenogenital syndrome.

Failure of the ovaries to produce estrogens (pre-puberal castration), like male eunuchoidism, gives rise to slight inhibition of bone development without growth impairment so that the end result is a eunuchoid gigantism. In contrast, bone development in Turner's syndrome is found to be normal or slightly retarded and there is usually pronounced dwarfism which has a genetic and not a hormonal cause.

In contrast to testosterone and anabolic steroids, estrogenic hormones have not been found to have any definite anabolic action, experimentally or therapeutically. They do not promote growth, tending rather to inhibit it, and probably promote bone development to a small extent. As in pseudosexual precocity due to a granulosa cell tumor, an apparently paradoxical effect is often seen when Turner's syndrome is treated with estrogens: not only does pubic hair develop but growth as a whole is also promoted (p. 720). In pituitary dwarfism it is only possible to achieve both actions with testosterone. Treatment with estrogens probably stimulates the production of androgenic adrenocortical hormones in the presence of an intact hypophysis (p. 1040).

c) Hormonal Control of Growth during Puberty

A period of accelerated growth begins at puberty, subsiding towards the end of puberty

and coming to a standstill with the fusion of the epiphyses. As has been shown in the preceding sections, this is also applicable to true precocious puberty and to precocious pseudopuberty associated with isolated hyperfunction of the gonads or adrenal cortex. Both androgenic gonadal hormones and androgenic adrenocortical hormones interact with STH to promote growth and bone development. The puberal growth spurt is apparently due to secretion of androgenic steroids and STH. In the absence of gonadal hormones, there is no spurt of growth during puberty, but a deficiency of adrenocortical androgens may also play a great part (p. 1038), particularly in girls. Because the epiphyses fuse late, the period of growth is prolonged, but the rate of growth is so slow in the last few years that the resultant gigantism is insignificant.

The earlier normal puberty or a pathologic form of puberty arises, the shorter is the total duration of growth and the smaller is the final size attained. The average size of women is less than that of men since the more rapid bone development in girls is paralleled by earlier puberty so that growth also ceases sooner.

C. Normal Values for Body Measurements and Development of Bones and Teeth

1. Height and Weight

Height and weight are the most important measurements for assessment of physical development, but at the same time it must not be forgotten that there are wide physiological variations. Heredity, standard of living and the acceleration of growth from one generation to another all have marked effects.

Normal percentile curves for boys and girls from birth to the 18th year are given in Figs. 4 and 5. The terminal point of the growth curve can be used to estimate the height in adults. Since weight continues to increase after growth has ceased up to the 30th year, special tables (Table 2) are necessary for the assessment of adult weights.

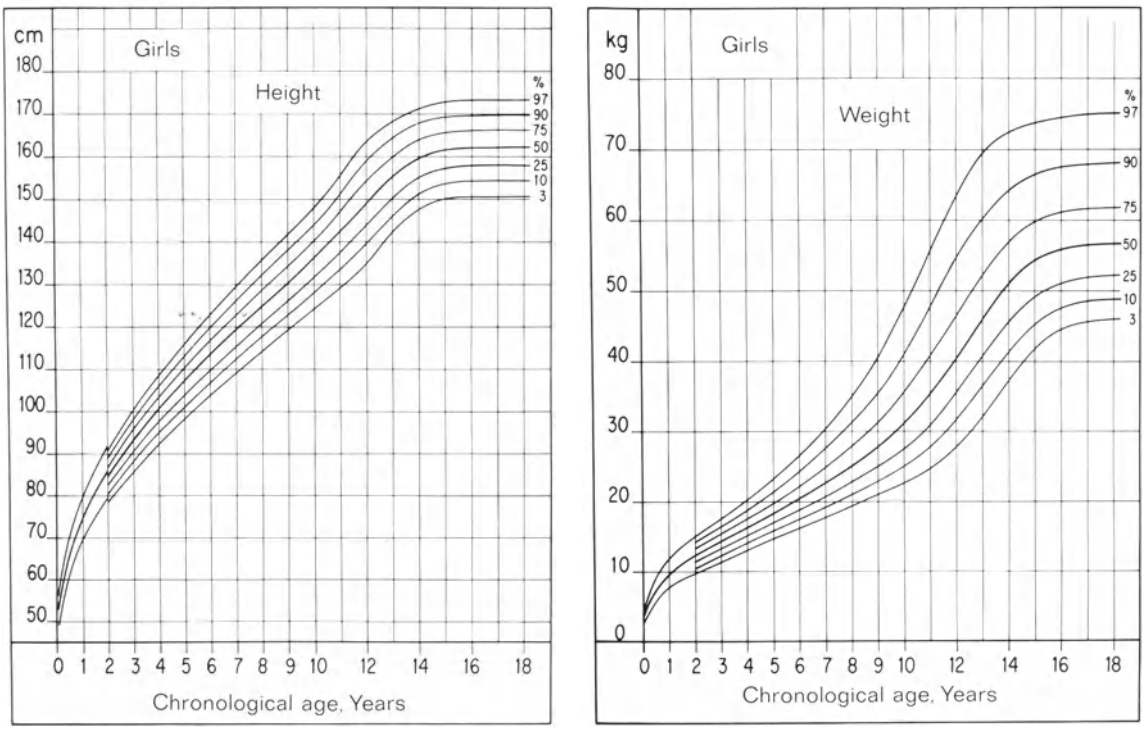


Fig. 5. Height and weight of girls. Percentile 50 represents median values. Percentile 10 (or 90) signifies that 10 % (or 90 %) of all subjects of the same age are shorter or lighter and 90 % (or 10 %) are taller or heavier

Height age and weight age signify the average age at which a certain size and weight are reached (percentile 50 in Figs. 4 and 5). A deviation of $\pm 20\,\%$ from the chronological age is completely normal and roughly corre-

sponds to the area of dispersion between percentiles 10 and 90.

If the average yearly increase in height and weight during the entire period of growth is considered (Fig. 6), the striking feature is the marked increase in height at the beginning of puberty. This spurt in growth does not occur in the absence of puberty.

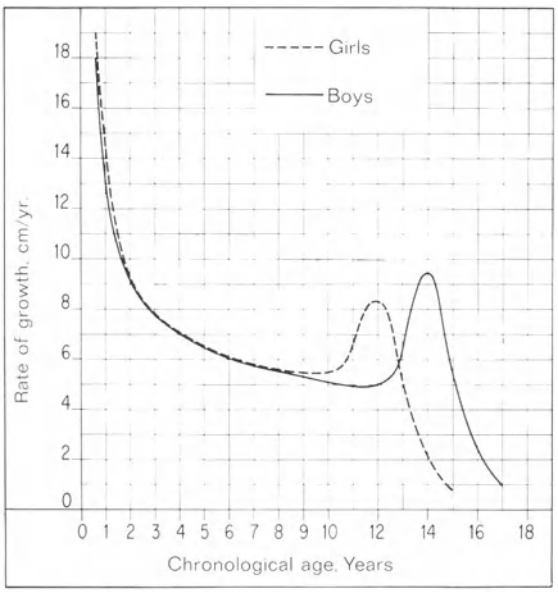


Fig. 6. Rate of growth (cm years) in a typical individual growth curve (TANNER, 1966)

2. Body Surface and Dosages for Tolerance Tests

Dosages per kg body weight used in tolerance tests given in this book are only applicable to adults. These dosages would be too low for children. During childhood, it is preferable to give the dose per m^2 body surface. The dosage according to body surface, however, can be used just as well in adults and can thus be employed more universally than the dosage according to body weight. The body surface can be assessed from body height and body weight with the aid of a nomogram. Experienced workers can estimate the body surface from the following rough scale of normal values: newborn = 3.5 kg = 0.2 m^2, 2 years = 12 kg = 0.5 m^2, 9 years = 30 kg = 1.0 m^2, adult = 65 kg = 1.7 m^2.

3. Body Proportions

As growth increases, the head becomes progressively smaller and the extremities progressively longer in proportion to the trunk. The relative lenghts of the trunk and the extremities is important in endocrinology. The simplest way of estimating these proportions is to measure the lower length or lower segment (height from floor to the upper edge of the pubic symphysis) and then calculate the upper length or upper segment (body height minus lower segment). The *sitting height* is very often measured instead of the upper segment and is only slightly different. The ratio of upper segment/lower segment is about 1.7 in the newborn and decreases with increasing age (Fig. 9). The ratio is about 1.0 in a 10-year-old. During puberty, the extremities are comparatively longer than at any other time of life so that the quotient may fall to 0.9 in boys and 0.95 in girls. It is slightly more than 1 in adult women and slightly less than 1.0 in men.

A rougher estimate of the proportion between trunk length and extremity length can be made by measuring the arm span (distance from finger tips of one outstretched arm to finger tips of the other outstretched arm) and body height. The arm span is slightly less than the body height before the 10th year of life and slightly more after the 10th year. It exceeds body height by a maximum of 4–7 cm in adults. The fact that arm span corresponds to height and the lower segment to the upper segment in the man is seen in LEONARDO DA VINCI'S famous artistic sketch of human proportions (Fig. 7).

The development of these proportions is retarded, i.e. the relative length of the extremities is too short (Fig. 8), in certain forms of dwarfism, particularly in hypothyroidism and chondrodystrophy. The ratio of upper segment/lower segment is higher than normal and the arm span shorter. The same is applicable to all forms of true and pseudosexual precocity after growth has ceased. In these patients epiphyseal fusion occurs before adult proportions are attained. Conversely, in other subjects the extremities are longer than normal, particularly in eunuchs and in arachnodactylia. This feature is less marked but still obvious in almost every case of delayed puberty. The ratio of upper segment/lower segment is then lower and the arm span longer than normal (eunuchoid stature).

4. Bone Development

Bone development is most easily assessed from an X-ray of the hand. Developmental age of the skeleton of the hand (bone age) can be determined by comparing the X-ray with Figs. 10–13. There are special atlases from which the precise bone age of the hand can be read (GREULICH-PYLE). Even more exact methods

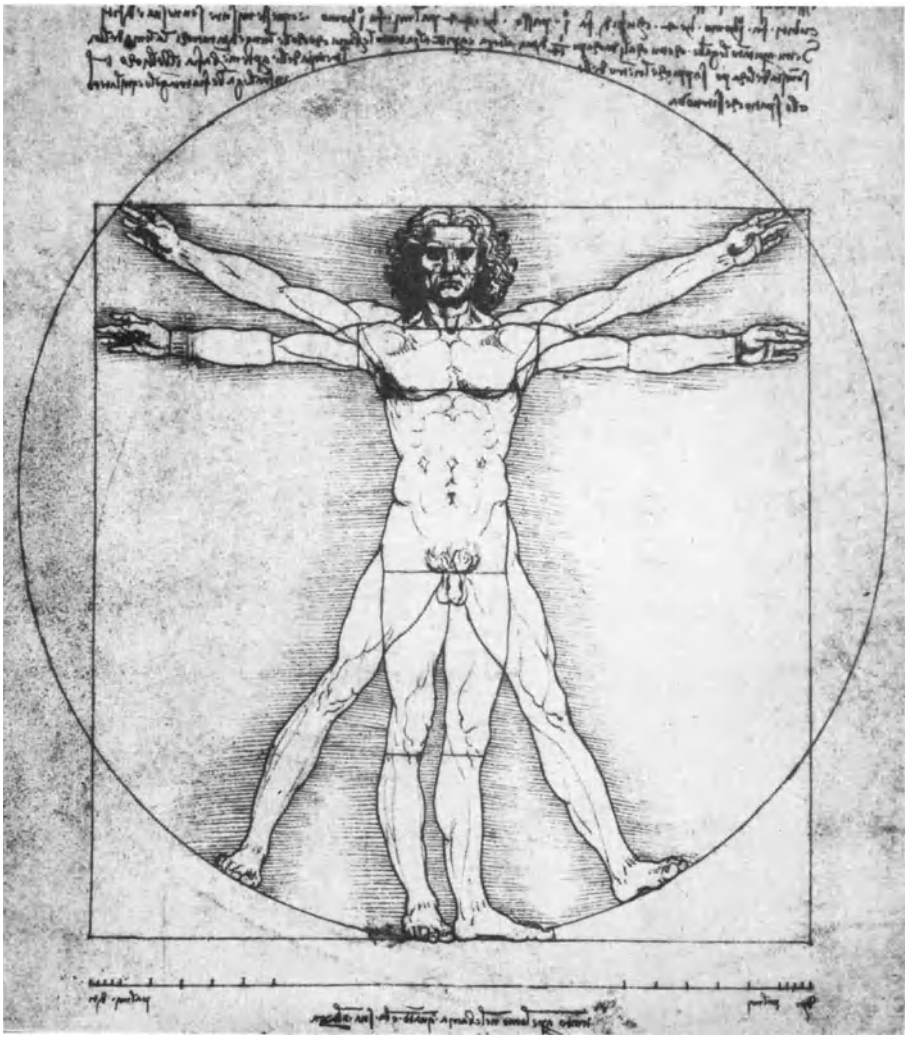


Fig. 7. LEONARDO DA VINCI'S sketch of the human proportions

are based on counting all the centers of ossification on one side of the body (SONTAG).

As in the case of height age and weight age, a deviation of about $\pm 20\%$ of bone age from chronological age is completely normal. Bone development is faster in girls than in boys, the greatest difference being found during puberty when bone development is about 2 years ahead in girls. It is not unusual to find a slight dissociation, i.e., a disturbance in the sequence in which the ossification centers arise. Marked dissociation is especially common in hypothyroidism and following severe generalized diseases.

Comparison of height age and bone age is valuable in the investigation of many growth disorders. In pituitary dwarfism (p. 98), height and bone age are retarded severely and to approximately the same extent. Bone age is more retarded than height age in untreated hypothyroidism during infancy (p. 165). Height age alone is advanced in hypophyseal gigantism (p. 107). Bone age is more advanced than height age in true and pseudosexual precocity (p. 1045).

In some endocrine disorders the X-ray of the hand is of value not only in the estimation of bone age but also in the assessment of bone structure. A marked calcified endplate of the radius and ulna with splintering of the ossification center of the ulna (epiphyseal dysgenesis) points to hypothyroidism (p. 173), whereas bone absorption in the region of the phalanges indicates hyperparathyroidism (p. 915). Generalized calcium deficiency is found in various disorders of calcium/phosphorus metabolism. Coarse ropelike structural changes are found in Turner's syndrome. Transverse lines in the vicin-

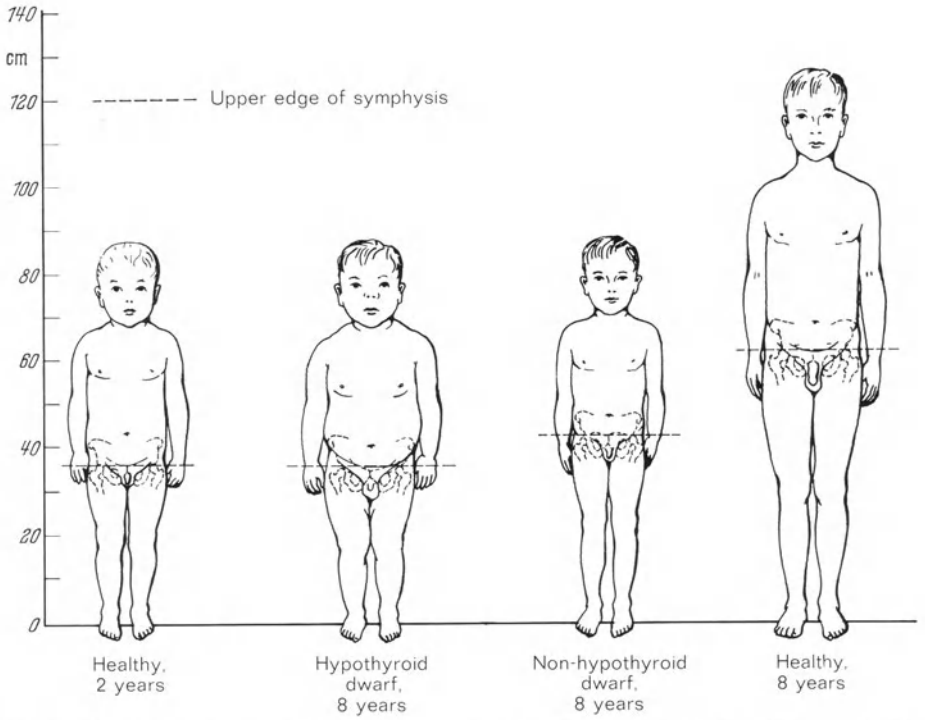


Fig. 8. Body proportions (upper segment/lower segment) in healthy subjects at ages of 2 and 8 years. In the middle, an 8-year-old hypothyroid dwarf whose proportions correspond to those of the healthy 2-year-old, and an 8-year-old non-hypothyroid dwarf with proportions appropriate to his true age. (According to WILKINS)

ity of the epiphyses in long bones and a coulisse-like structure of the ossification centers (Fig. 10, p. 173) can indicate a sudden change from slow to rapid bone growth even months to years after a temporary inhibition of growth in generalized illness or the onset of treatment with thyroid extract in hypothyroidism.

5. Prognosis of Puberty and Growth from Body Height and Bone Development

Bone age is a much more reliable indicator of the time puberty will occur than chronological age or body measurements. The appearance of the first sesamoid bone of the thumb (Fig. 11) at a bone age between 10–11.5 years in girls and between 12.5–13.5 in boys usually coincides with the onset of puberty. Menarche and the point at which mature spermatazoa are probably produced for the first time usually occur about two years later.

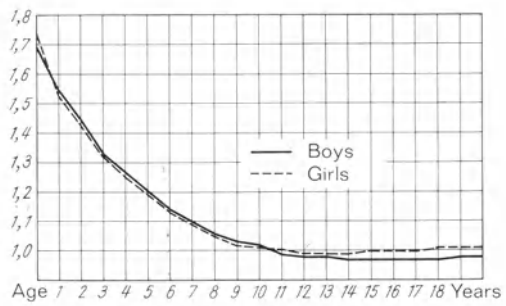


Fig. 9. Mean values for ratio of upper segment/lower segment. (After ENGELBACH, 1932)

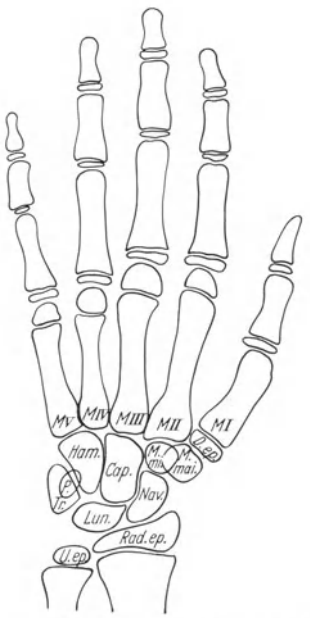


Fig. 10. Skeleton of the hand of a 12-year-old boy (KspZ)

This allows a rough prediction of the time puberty will occur after the 8th year. Thus for example, if bone age is two years in advance of chronological age in a 9-year-old boy puberty can be expected at the age of 11 instead of at 13.

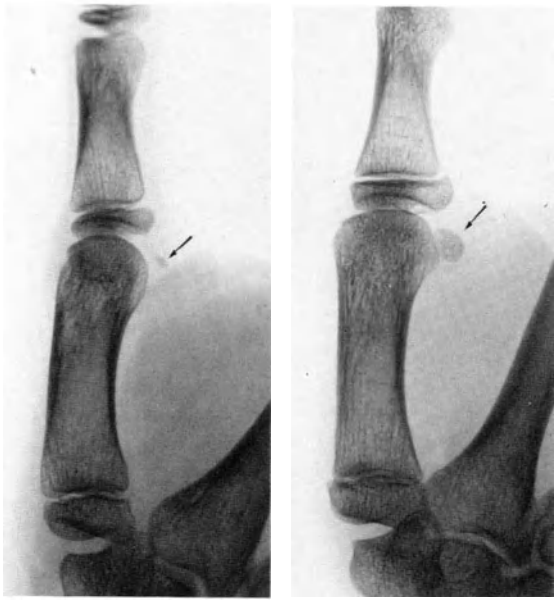


Fig. 11. First sesamoid bone of the thumb shown on the X-ray. Left, in a boy at the age of 13; right, in a boy of 15 (KspZ)

Since growth ceases in girls at a bone age of 16–17 years and in boys at a bone age of 18–19, the further course of growth can be roughly predicted from body height and bone age in children with a bone age of at least 6 years (Table 3). However, this necessitates a completely accurate assessment of bone age, which can be obtained with the aid of the *Greulich-Pyle Atlas*. This possibility is of importance since parents of children growing too slowly or rapidly despite the absence of any endocrinological abnormality are particularly likely to demand a prognosis on growth from the doctor.

6. Dental Development

Development of the teeth is best assessed from a lateral X-ray of the lower jaw. The developmental age of the teeth (dental age) can be assessed by comparing the degree of mineralization of the teeth with Fig. 14. Fig. 14 also shows hypoplasia of the enamel of the permanent incisors is caused by a disorder ot calcification during very early childhood (rickets, hypoparathyroidism).

Table 3. Table for predicting adult height from body height and skeletal age. The figures indicate the percentage of final adult height at a given skeletal age. (From BROCK after tables by BAYLEY and PINNEAU)

Skeletal age in years and months	Boys			Girls		
	Skeletal age			Skeletal age		
	advanced	normal	delayed	advanced	normal	delayed
6. 0			68.0		72.0	73.3
6. 6			70.0		73.8	75.1
7. 0	67.0	69.5	71.8	71.2	75.7	77.0
7. 6	68.5	70.9	73.8	73.2	77.2	78.8
8. 0	69.6	72.3	75.6	75.0	79.0	80.4
8. 6	70.9	73.9	77.3	77.1	81.0	82.3
9. 0	72.0	75.2	78.6	79.0	82.7	84.1
9. 6	73.4	76.9	80.0	80.9	84.4	85.8
10. 0	74.7	78.4	81.2	82.8	86.2	87.4
10. 6	75.8	79.5	81.9	85.6	88.4	89.6
11. 0	76.7	80.4	82.3	88.3	90.6	91.8
11. 6	78.6	81.8	83.2	89.1	91.4	92.6
12. 0	80.9	83.4	84.5	90.1	92.2	93.2
12. 6	82.8	85.3	86.0	92.4	94.1	94.9
13. 0	85.0	87.6	88.0	94.5	95.8	96.4
13. 6	87.5	90.2		96.2	97.4	97.7
14. 0	90.5	92.7		97.2	98.0	98.3
14. 6	93.0	94.8		98.0	98.6	98.9
15. 0	95.8	96.8		98.6	99.0	99.4
15. 6	97.1	97.6		99.0	99.3	99.6
16. 0	98.0	98.2		99.3	99.6	99.8
16. 6	98.5	98.7		99.5	99.7	99.9
17. 0	99.0	99.1		99.8	99.9	100.0
17. 6		99.4		99.95	99.95	
18. 0		99.6			100.0	
18. 6		100.0				

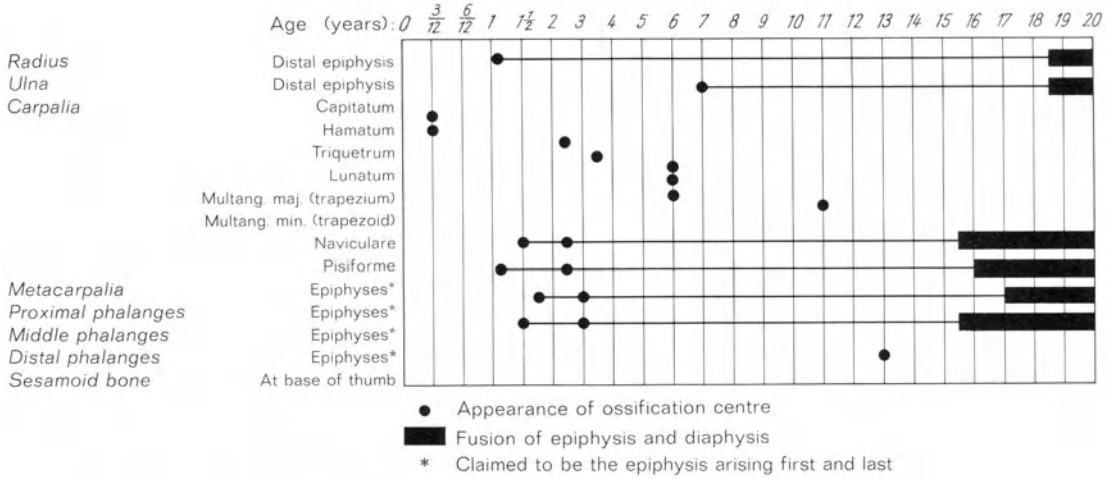


Fig. 12. Development of the skeletal structures of the hand in the male sex

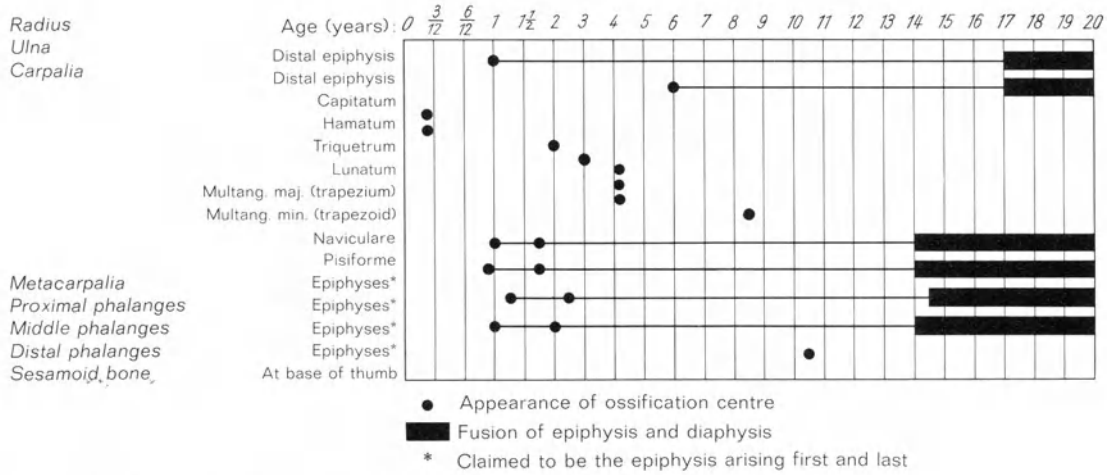


Fig. 13. Development of skeletal structures of the hand in the female sex

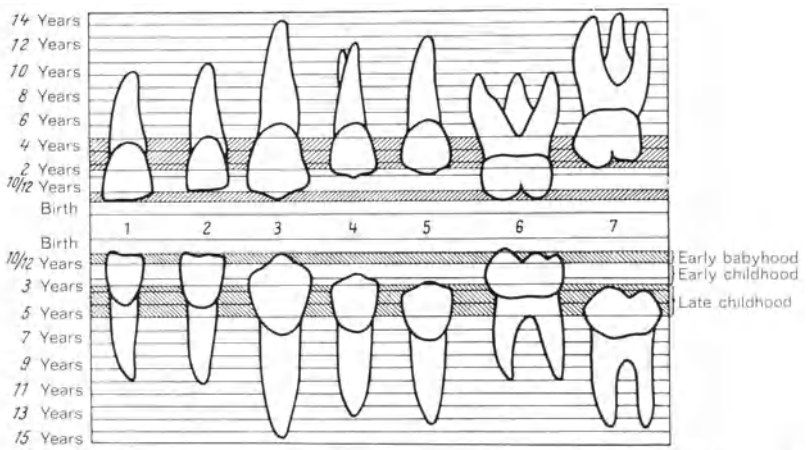


Fig. 14. Radiologically detectable mineralization of permanent teeth. (After MASSLER)

Development of the teeth usually deviates less from the normal than bone development in endocrine disorders. Thus, dental age is less retarded than bone age in infantile hypothyroidism (p. 165) and in pituitary dwarfism (p. 98). In contrast, dental age is normal or only slightly advanced in all forms of pseudo- or true precocity, although bone age is extremely advanced (Fig. 29). Development of teeth is obviously less dependent on endocrine factors than bone development. Hypoparathyroidism arising during infancy is an exception (p. 895), since development of the teeth is often considerably impaired due to deficient calcification of the roots. The X-ray is valuable in the diagnosis of hyperparathyroidism (p. 915) since it reveals the absence of the lamina dura (cortex of the dental alveoli).

7. Graphic Representation of Growth and Development

Fig. 9 on p. 172 and Fig. 29 on p. 1047 show a simple and clear graphic representation with chronological age plotted along the abscissa and developmental age along the ordinate. The curve appropriate to each developmental age (height age, weight age, bone age, mental age, etc.) is then drawn in. The different measures of development can then be determined at a glance.

D. Disorders of Growth

1. Definition of Dwarfism and Gigantism

Any definition of small and large stature and dwarfism and gigantism is subjective and therefore none can claim to be standard.

During childhood, the concepts of small and tall stature are used (Chap. XVIII) when the height age deviates by more than 20% from the norm, and those of dwarfism or gigantism when the deviation exceeds 40%. According to another definition, dwarfism or gigantism is present if the height differes more than three standard deviations from the normal mean. Both definitions incorporate almost the same limits during childhood. If the same form of measurement is applied to adults, dwarfism (or small stature) is present in a male if he is smaller than 145 cm (or 165 cm), and gigantism (or large stature) if he is taller than 200 cm (or 185 cm). Similarly, dwarfism (or small stature) is present in a woman if she is smaller than 135 cm (or 155 cm) and gigantism (or tall stature) present if she is taller than 185 cm (or 170 cm).

Table 4. Etiological classification of dwarfism

1. *Deficiency of building materials*
Hypocaloric dwarfism
Intestinal dwarfism (see under 3)
2. *Neural and hormonal disorders*
Hypothalamus and pituitary
Dyscerebral and microcephalic dwarfism, Laurence-Moon-Biedl-Bardet syndrome
Constitutional retardation of growth and puberty
True precocious puberty
Pituitary dwarfism
Thyroid
Hypothyroid dwarfism
Pancreas
Badly stabilized diabetes mellitus
Adrenals
Cushing's syndrome
Gonads
Pseudosexual precocity[a] with gonadal tumors
3. *Nonhormonal metabolic disorders*
Renal dwarfism
Renal malformations, chronic nephritis, Fanconi syndrome, renal diabetes insipidus, etc.
Intestinal dwarfism
Celiac disease, cystic fibrosis, megacolon, etc.
Hepatic dwarfism
Glycogen storage disease, cirrhosis, etc.
Anoxemic dwarfism
Congenital heart disease, bronchiectases, chronic anemia, etc.
Rachitic dwarfism
Vitamin-D deficiency and vitamin D-resistant rickets
Lipoidosis
Gaucher's disease, Niemann-Pick, Hand-Schüller-Christian, and Pfaundler-Hurler's disease
4. *Deficient growth potential of bones*
Primordial dwarfism and progeria
Congenital skeletal disorders
Achondroplasia, dysostosis (d. multiplex Pfaundler-Hurler, d. enchondralis Morquio, d. cleidocranialis, etc.)
Acquired skeletal disorders
Rickets, hyperparathyroidism
Congenital dysmorphic syndromes
Gonadal dysgenesis, pseudohypoparathyroidism, autosomal chromosomal disorders (Down's syndrome, etc.)

[a] Growth retardation only after epiphyseal fusion.

2. Etiological Classification of Dwarfism

A survey of the various forms of dwarfism is presented in Table 4. The etiological classification corresponds to the growth factors given on p. 1015. The different forms of dwarfism due to endocrine disorders are discussed in the appropriate chapters dealing with the organs and on p. 1031. This chapter deals with the nonhormonal forms of dwarfism which are frequently misinterpreted as endocrinopathies and the differential diagnosis of dwarfism.

3. Dyscerebral and Microcephalic Dwarfism

Microcephalia, brain malformations, and congenital and acquired brain damage of all types are often connected with dwarfism. A deficiency of growth hormone is only exceptionally found to be the cause in such cases. This suggests that cerebral and hypothalamic factors can also influence growth and development via nonhormonal routes.

4. Hypocaloric and Psychosocial Dwarfism

Numerous studies on growth in school children during war and postwar times have shown that deficient nutrition definitely impairs growth and that proper nourishment promotes growth in undernourished children. The best examples of hypocaloric dwarfism can be found among incorrectly and poorly fed babies, children with severe chronic generalized illnesses, and children with anorexia persisting over many years. These last patients illustrate extremely well that psychological influences can also interfere with normal growth by their effect on nutritional uptake.

In addition to children in whom undernourishment or long-standing severe psychological conflicts are apparent there are others with dwarfism, striking emaciation and slightly retarded bone development. In these children, a deficient diet is only discovered to be the cause of the dwarfism after change of environment and an optimal diet have produced an acceleration in growth.

In the U.S.A. there has recently been a special study of forms of dwarfism due to a combination of psychological and social factors. These types of dwarfism are usually reversible (PATTON, 1962; POWELL, 1967), and are referred to as "emotional deprivation" or "maternal deprivation" or as psychosocial dwarfism. Apart from the dwarfism, behavioral disturbances and neglect are also dominant features. It is not quite certain whether psychic factors can inhibit growth directly or whether the dwarfism is connected with the malnutrition. The latter appears more probable. In some cases the concentration of growth hormone in the blood was found to be low and to respond insufficiently to stimulation. A functional, i.e. reversible, growth-hormone deficiency may therefore be present. The growth hormone concentration is, however, raised in other cases of more acute undernourishment with protein deficiency (Kwashiorkor) (PIMSTONE, 1966). If the child can be successfully removed from this situation by a change of environment or improved diet, accelerated compensatory growth can be observed with complete correction of the condition. If it is left untreated, the growth curve is similar to that in patients with growth-hormone deficiency (p. 98) or to that found in constitutional delay of growth and puberty, discussed in the following section.

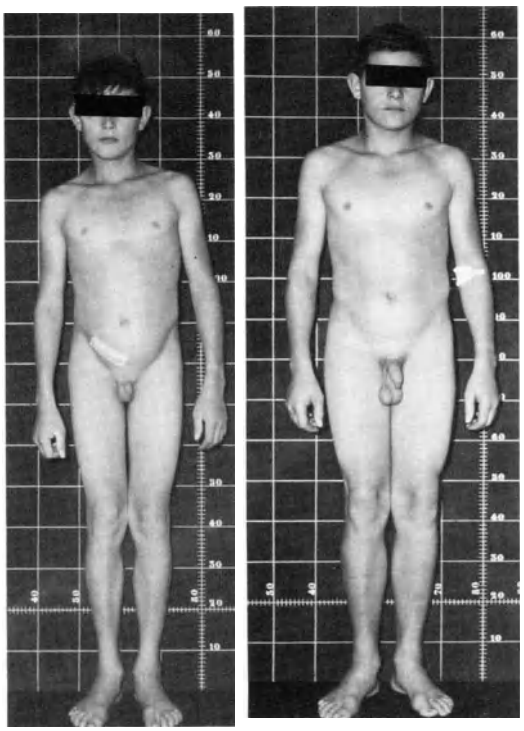


Fig. 15. Familial constitutional delay of development with spontaneous puberty at the age of 18. Left, boy of almost 18 with definite dwarfism, puberty still absent, slightly eunuchoid proportions. Bone age of almost 13 years signifies that puberty is imminent. Right, exactly one year later. As expected, puberty occurred spontaneously and there was a marked acceleration in growth (PRADER, 1955)

5. Constitutional Delay of Growth and Development

Bone development and puberty are retarded in many children with dwarfism in whom no pathologic findings and no proof of inadequate nutrition can be detected. During shool age, height, weight, and bone development are usually retarded by 2–4 years. Puberty and the puberal spurt in growth occur somewhat later than in other children, between the 14th and 18th years, but are absolutely normal. As the period of growth is prolonged to the 20th to 23rd year of life these individuals reach a normal height, even if at the lower normal limits, at a later date.

This delay in growth and puberty is the most common cause of dwarfism in childhood. It is usually familial. The case history may

reveal that the father was also one of the smallest at school and was still growing at the age of 20, or that menarche was late in the mother, occurring at the age of 15 or 16, or that puberty developed very late in older siblings and they only started to catch up on growth after this.

The clinical diagnosis is based on the following elements: the child or youth gives a healthy and robust impression, there are no signs of endocrinopathy, the growth curve lies below and almost parallel to that of the 3rd percentile, bone age is retarded to the same extent as growth, the family history may reveal similarly retarded growth and puberty in the father or mother or older siblings. In such circumstances detailed endocrinological examinations are not indicated. If, however, the growth curve deviates further from the 3rd percentile, isolated growth hormone deficiency must be considered in the differential diagnosis and appropriate tests initiated.

The ability to predict from the bone age when puberty can be expected (p. 1024) and what the future adult height will be (p. 1024) is crucially important in the selection of *treatment*. In order to leave endogenous development of puberty undisturbed, every type of hormonal therapy should theoretically be postponed until the bone age (girls about 11.5 years, boys about 13.5 years) at which the first signs of puberty should appear is reached. When these do not appear as expected the diagnosis of constitutional growth and developmental retardation must be discarded and a true hypogonadism assumed as the cause. As a general rule it is permissible to delay treatment if puberty is almost imminent. But after the 15th year or sometimes even earlier, a true psychological emergency may arise. During confidential talks with the patient it suddenly becomes apparent how inadequate he feels physically and often socially, how difficult it is for him to keep up in sports, how awkward, inferior, and abnormal he feels, how he is made fun of and terrorized by his associates and how he lives in a constant state of fear. This situation presents a pressing psychosocial indication for temporary treatment with chorionic gonadotropin or testosterone. Chorionic gonadotropin has the advantage that any expected response of the testes to stimulation can soon be recognized as secondary sexual characteristics develop quickly. A weekly injection of 2000 units for 3 months is a suitable dosage. Treatment with testosterone is correct either from the beginning or subsequent to administration of chorionic gonadotropin (10–25 mg methyl testosterone daily orally, or 250 mg of a depot testosterone preparation given by i.m. injection each month). Testosterone, however, should only be given until a bone age of 13.5 years has been reached. Body weight, height, and physical strength will increase rapidly during treatment. Secondary sexual characteristics appear at the same time but the testes remain small in size since endogenous gonadotropin production is inhibited by the treatment. Appetite, efficiency, and self-confidence also increase. Puberty continues spontaneously under the control of endogenous gonadotropins after treatment is discontinued, and the testes rapidly increase in size providing the bone age has passed the stage of 13–13.5 years.

6. Primordial Dwarfism

In primordial dwarfism, body proportions, bone development, development of puberty and the age at which growth stops correspond roughly to the chronological age. In other words, with the exception of growth in height and weight, these children develop absolutely normally and are capable of reproduction. The incidence of this form of dwarfism is both sporadic and familial. Growth is usually retarded at the time of birth in spite of a pregnancy of normal duration (Fig. 17). It is not unusual for subjects in whom the condition is sporadic to have children of normal size. In other cases, the family tree shows a true selection for dwarfism, since small people prefer to choose small marriage partners and they in turn have small children.

7. Progeria

Progeria (Hutchinson-Gilford syndrome) is a very rare, non-familial, congenital growth disorder with premature aging. The patients are of normal intelligence and all have exactly the same unmistakable appearance. As well as dwarfism and deficient panniculus, the condition is characterized by an old-looking bird-like face (Fig. 16).

Typical symptoms usually arise as early as the first year of life. Growth is greatly retarded but ceases at the normal time. Average height is only 117 cm and the mean weight only 16.5 kg at the age of 18 (Thomson, 1950). The face is strikingly small compared to the skull, which has a slightly hydrocephalic appearance. The eyes are prominent and the nose is beak-like and pronounced. The lower jaw is hypoplastic. Hair on the head, eyebrows, and eyelashes is usually sparse or completely absent. Veins on the forehead are greatly dilated and protruding. X-rays show a mild calcium deficiency in the bones, coxa valga,

and hypoplasia of the distal ends of the clavicular. Extremities are always held in a position of slight flexion. Arteriosclerotic and arthrotic changes always arise at a very early age. Death usually occurs during the 2nd decade and is most often due to coronary insufficiency. The oldest known case reached the age of 26 years.

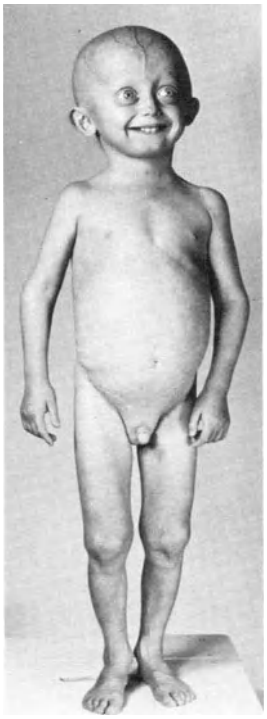


Fig. 16. Classic progeria in a $3\frac{1}{2}$-year-old boy. Body height is 85 cm instead of 95 (Rossi, 1951)

The cause of progeria is unknown. Some time ago hypophyseal insufficiency was thought to be the cause, but this has never been proved. More recently, hereditary mesenchymal dysplasia with resistance to growth hormone and insulin has been postulated (Villee, 1969).

8. Dysmorphic Syndrome with Dwarfism

The numerous syndromes of dwarfism with multiple dysmorphic features or multiple malformations are extremely difficult to diagnose and even more difficult to treat. The regions predominantly affected by the multiple malformations are those of the head, face, hands, feet, heart, and external genital organs. Dwarfism is commonly already present at birth. Bone development corresponds to the chronological age or is retarded. Intelligence is normal or below normal depending on the type of syndrome. Turner's syndrome (gonadal dysgenesis, p. 713), pseudo-Turner's syndrome (p. 719), pseudohypoparathyroidism (p. 899), autosomal chromosomal disorders of which mongolism (Down's syndrome) is the most frequent, and numerous other syndromes belong to this group. The diagnosis is important for prognostic reasons and for genetic counselling, but it requires special knowledge in this field (Smith, 1970) and cannot be discussed any further at this point.

9. Clinical Investigation of Dwarfism

The survey of the various forms of dwarfism (Table 4 on p. 1027), the types of growth in Fig. 17, and the summary of the differential diagnosis in Table 5 should be kept in mind during the following account.

Clinical investigation of dwarfism is often laborious and time-consuming. The cause can only seldom be detected at first glance (hypothyroidism, chondrodystrophy, mongoloid idiocy, etc.). An exact family history (height of parents and siblings, age at onset of puberty, and at cessation of growth) and a careful personal history giving information about the course of growth until the present time, nutrition, appetite and previous illnesses are absolutely essential. Thorough general physical examination with urinalysis is also of the utmost importance. It is very important to record height, weight, bone age, body proportions and the state of puberal development to provide a standard of comparison in assessing the subsequent course of growth and development at later examinations. The X-ray of the hand is used to assess bone age and also reveals any metabolic and endocrine skeletal disorder.

Only tests aimed at confirming a suspected diagnosis should actually be performed; even consideration for the patient alone forbids indiscriminate testing for all conditions which can cause dwarfism.

A thorough case history and clinical examination nearly always lead to the diagnosis in adults. The diagnosis is more difficult in children but far more important for prognostic and therapeutic reasons. Often, however, even the most careful investigations do not definitely reveal the cause of the dwarfism in the child. Periodic observation is very valuable since the course of growth is quite characteristic for most forms of dwarfism.

If investigations do not reveal an easily detectable endocrinopathy (hypothyroidism, Cushing's syndrome), or metabolic disorder, cerebral disturbance or skeletal disorder, and the child gives a generally healthy impression and appears to be of normal intelligence, the condition is frequently due to constitutional

retardation of growth and puberty (p. 1028). Less commonly, it may be primordial dwarfism (p. 1029), pituitary dwarfism (p. 98) or Turner's syndrome (p. 713) without striking dysmorphic features. Pituitary dwarfism can be detected by means of thorough examination of pituitary function with estimations of growth hormone. Turner's syndrome must be condidered in every girl of small stature, and pterygium etc. must be looked for carefully. A negative sex

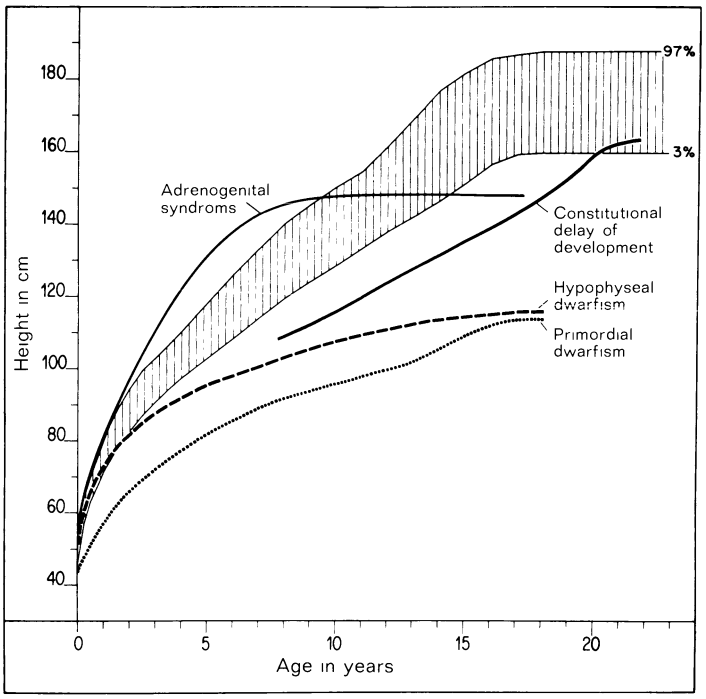


Fig. 17. Typical growth curves in various forms of dwarfism (PRADER, 1971)

Table 5. Differential diagnosis of endocrine dwarfism in childhood

Diagnosis	Growth	Bone maturation (and puberty)	Intelligence	Special features
Dyscranial dwarfism	Delayed	Delayed	Reduced	Microcephalia Neurological findings possible
Hypocaloric dwarfism Psychosocial dwarfism	Delayed	Delayed	Normal	History Emaciation
Constitutional delay of growth and puberty	Both similarly delayed		Normal	Parental history Healthy
Primordial dwarfism	Delayed	Normal or slightly delayed	Normal	Parents often very small Healthy
Pituitary dwarfism				
a) isolated deficiency of growth hormone	Very delayed	Less delayed than growth	Normal	Doll-like appearance Normal body proportions
b) deficiency of several pituitary hormones	Both similarly delayed		Normal	As in a) associated with other hormonal failures
Hypothyroidism	Delayed	More delayed than growth	Reduced	Characteristic appearance Infantile body proportions
Cushing's syndrome	Delayed	Delayed	Normal	Obesity with other Cushing symptoms
Turner's syndrome (gonadal dysgenesis)	Very delayed	Less delayed than growth	Normal or slightly reduced	Typical dysmorphic characteristics
True and pseudo-precocious puberty	Accelerated up to 12th year, then standstill with dwarfism	Greatly accelerated	Normal	Accelerated somatic development premature secondary sexual characteristics

chromatin confirms the diagnosis whereas a positive sex chromatin does not help and demands analysis of the chromosomes when Turner's syndrome is clinically suspected.

10. Prognosis and Treatment of Dwarfism

The *prognosis for growth* is of greatest practical importance in children. If an endocrine hypofunction is present the prognosis will depend on regular substitution therapy. The time at which puberty will occur and the future adult height can be predicted quite accurately in all other forms from body height and bone age (p. 1024). Obviously no further growth is possible after fusion of the epiphyses.

Causal treatment is possible in only a few cases of dwarfism. It includes optimal nutritional intake if the condition is due to malnutrition, administration of human growth hormone in pituitary dwarfism, or of thyroxine in hypothyroidism, insulin treatment in diabetes mellitus, surgical removal of endocrine-active tumors, treatment with cortisone in cases with the adrenogenital syndrome and inhibition of gonadotropins in cases of sexual precocity.

Some of the growth-promoting drugs recommended for *symptomatic treatment* are:

1. Pituitary Growth Hormone. Extracts from animals are useless. The amounts of growth hormone extracted from human pituitary glands at our disposal are barely adequate for the treatment of pituitary dwarfism, so that we have very little experience of its action in other forms of dwarfism. The usual doses currently used for symptomatic treatment (2–6 IU 2–3 times weekly) appear to have little or no effect.

2. Thyroid Hormones. The growth-promoting effects of these hormones are very slight except in hypothyroidism. This form of treatment must be used with caution since it involves the risk of accelerated development of the bone age.

3. Testosterone. Testosterone promotes growth considerable. Its androgenic action is a disadvantage and only very low doses can be used before puberty. Another drawback is its stimulating action on bone maturation. Since bone maturation is promoted more than growth (p. 1020), there is a risk of premature epiphyseal fusion with premature arrest of growth as with an overproduction of endogenous androgens (p. 713). In other words, the acceleration of growth is obtained at the expense of a reduction in future adult height. Testosterone should therefore only be given after the 12th year of age and only to boys with a good growth prognosis. Symptomatic use of testosterone has been discussed in connection with the treatment of constitutional retardation of growth and puberty (p. 1029).

4. Anabolic Steroids. It has been claimed that the growth-promoting anabolic action of various synthetic testosterone derivatives is much greater than their androgenic effect. In practice, however, it has been found that, like testosterone, they usually have stimulating and androgenic effects on bone maturation in doses promoting growth. The misgivings applicable to testosterone are thus also applicable to these drugs. Exceptional caution is required, particularly in treating girls, because of the androgenic action.

5. Insulin. Findings from animal experiments (p. 1019) and theoretical consideration suggest that symptomatic promotion of growth may be possible with insulin. Its use, however, is problematic because of the danger of hypoglycemia and there is no practical experience as yet.

6. Nutrition. An optimal intake of calories and proteins is advisable in every form of dwarfism. Definite success can only be expected if the inhibition of growth is due to deficient nutrition. Trying to force anorexic children to eat is usually useless and only leads to psychoreactive disturbances.

7. Vitamins, Liver Preparations, Antibiotics and Antiallergics. It has been claimed that vitamin B_{12}, liver extracts, certain antibiotics and, more recently, certain antiallergic drugs can promote growth directly or by stimulation of the appetite. There is no definite evidence of an action of this kind in man. Even if a clinician is sceptical about their validity, trials with these drugs are occasionally indicated for psychological reasons. Vitamins and liver preparations are then preferable, and the use of actual pharmaceutical preparations is only justifiable if regular check-ups are carried out.

11. Tall Stature and Gigantism

The various forms of gigantism are presented in Table 6, in which the same etiological classification has been used as for dwarfism (Table 4). The different forms of gigantism due to endocrine factors are dealt with in the corresponding chapters. A few nonhormonal forms of gigantism which are still frequently misinterpreted as endocrinopathies are discussed below with the differential diagnosis.

Table 6. Etiological classification of gigantism

1. *Excessive building materials*
Prepubertal and pubertal obesity (subsequent to pituitary hyperfunction)
2. *Neural and hormonal disorders*
Hypothalamus
Dyscerebral gigantism with hydrocephalus, etc.
True precocious puberty
Hypophysis
Hypophyseal gigantism
Thyroid
Hyperthyroid gigantism
Adrenals
Adrenogenital syndrome
Gonads
Pseudo-precocious puberty with gonadal tumors
3. *Nonhormonal metabolic disorders*
Arachnodactylia (Marfan syndrome)
Homocystinuria
4. *Excessive growth potential of bones*
Primordial gigantism
XYY syndrome
Wiedemann-Beckwith syndrome with omphalocele and macroglossia

In *cerebral gigantism*, gigantism is present from birth or babyhood. The striking features are: the large dolichocephalic head with the high forehead and large orbits, large plump hands and feet, often mild mental deficiency and generalized motor clumsiness. Bone age and puberty are usually somewhat advanced. The EEG is frequently found to be pathologic and there is often slight hydrocephalus. The syndrome occasionally arises among siblings. Hormonal findings correspond to the developmental age. Contrary to expectation, growth hormone levels are not raised. The pathogenesis is just as obscure as is that of microcephalic dwarfism.

There is also a *primordial form of gigantism*. Apart from the conspicuous height, these individuals are healthy and normal in every respect. Bone maturation and development of puberty are within normal limits. This form of gigantism is frequently familial. For understandable psychological reasons, very tall individuals often marry each other, so that as in dwarfism, there is true selection of gigantism.

The *differential diagnosis* is seldom difficult. The general examination and family history usually allow a definite diagnosis to be made. In the presence of impaired vision, and disorders of the nerves supplying the ocular muscles, the rare diagnosis of a pituitary tumor would have to be considered. The common XYY syndrome (p. 468) must be considered in cases where youths are extremely tall compared with their parents. The diagnosis is confirmed by means of Y-fluorescence staining of the buccal smear or by direct chromosomal investigation.

The *prognosis* about when puberty will occur and the future adult height can easily be determined from the age, height and bone age.

Causal treatment is only possible in the rare hormonal disturbances. There is no causal therapy available for the other types or for constitutional gigantism, which is very common. During school age and adolescence, extreme body height often leads to serious psychological problems and to organic kyphosis (Scheuermann's disease) or to psychogenic posture kyphosis. If the expected adult height is extreme, symptomatic treatment to reduce the future height is desirable. An urgent psychosocial indication for this type of treatment may present particularly in girls. As in precocious puberty, high doses of sexual hormones can reduce the phase of growth and thus the expected adult height (in girls, estrogen medication with gestagens every 4th week, testosterone in boys). Therapy of this kind inhibits gonadotropin secretion and thus suppresses gonadal development, at least temporarily, but current knowledge of the optimal dose and the complications associated with the treatment is not sufficiently advanced for it to be recommended for general use.

E. Puberty

1. Survey

Puberty is the phase of development at which secondary sexual characteristics arise and the gonads attain incretory and secretory maturation. It begins with the development of the first secondary sexual characteristics, generally lasts for about 5 years and ends when fertility has been attained shortly before cessation of body growth. Occasionally, puberty is defined as the first phase of development up to menarche (roughly the same period of time in boys), and the term adolescence is applied to the subsequent phase of development up to termination of growth. No distinction is usually made now, however, between puberty and adolescence. On average, puberty arises almost two years earlier in the female than in the male sex. Time relations vary according to race and other hereditary factors and are also influenced, like growth, by exogenous factors, way of life and nourishment. In addition, an earlier maturation (pubertal acceleration) has become obvious over several generations, which is presumably also related to exogenous factors.

The onset of puberty is now between the ages of 8 and 14 years in girls, and between 10 and 16 years in boys. Girls are sexually mature and adult between the ages of 14 and 16 years, and boys between 16 and 20.

The reader is referred to the chapters on the ovary and testis for the histology of gonadal maturation. Only external characteristics of puberty and the underlying hormonal relations are discussed in this section.

2. External Characteristics and Course

Tables 9 and 10 show the average course of puberty in girls and boys. Tables 7 and 8 show the usual stages used today for the development of pubic hair and breasts. Fig. 21 gives the normal values for testicular volume. The development as a whole shows a wide chronological variation between individuals (Figs. 18 and 19), whereas the order in which the individual characteristics arise and the time intervals between them vary much less.

As described in the discussion of growth (p. 1015), the *spurt of growth during puberty*, the *bone maturation* leading to fusion of the epiphyses and *standstill of growth* are the main characteristics of puberty. Bone development is related much more closely to puberty than chronological age or body size. This makes it possible to predict the onset of puberty from the chronological age and bone age. The relations between puberty and bone development and between bone development and arrest of growth also explain why growth comes to a halt sooner with early puberty. This rule, however, only applies to individual development and not to the generalized acceleration of growth and puberty observed in modern children. Puberty occurs at an earlier age now than in earlier generations and children, therefore, now reach adulthood earlier, but even so they are also taller than children of past generations. This increase of body height in spite of earlier puberty can be explained by the fact that the more intense prepubertal growth more than compensates for the shorter duration of growth.

Table 7. Stages of breast development. (After TANNER, 1962)

B 1	No palpable glands
B 2	Breast bud: areola enlarged, protusion of glands in region of areola
B 3	Gland larger than areola
B 4	Budding breast: glands in region of areola become elevated separately from the other glands
B 5	Mature breast: retraction of the areolar protusion into the general contour of the breast

Table 8. Stages of pubic hair. (After TANNER, 1962)

P 1	No hair
P 2	A little pubic hair around root of penis or on labia majora, unrecognizable on photo of whole body
P 3	Dense hair in circumscribed limits, visible on photo
P 4	Dense hair as in adult but less extensive
P 5	Extensive dense hair with horizontal upper margin and extending laterally onto thighs
P 6	Triangular extension towards umbilicus

Table 9. Chronological table of the development of puberty in girls

Age (years)	Physical characteristics
Before 8	Infantile state
10–11	Breast buds = thelarche (B 2) Acceleration of growth Maturation of vaginal mucosa
11	First pubic hair = pubarche First sesamoid bone of thumb
11–12	Marked growth of external and internal genitalia
12–13	Pubic hair and breast development in Stage 3 Peak height velocity
13	Menarche, irregular anovulatory menstruation Axillary hair Pubic hair and breast development in Stage 4
14–15	Regular ovulatory menstruation Pregnancy possible Pubic hair and breast development in Stage 5
16–17	Epiphyseal fusion and standstill of growth

Table 10. Chronological table of the development of puberty in boys

Age (years)	Physical characteristics
Before 10	Infantile state
11–12	Testes begin to grow
12–13	Appearance of pubic hair = pubarche Enlargement of penis starting Acceleration of growth
13–14	Marked growth of testes and penis Pubic hair in Stage 3 Mild swelling of breasts First sesamoid bone of thumb
14	Peak height velocity
14–15	Moustache appearing on upper lip Pubic hair in Stage 4 Axillary hair Marked breast swelling
15–16	Breaking of voice Pubic hair in Stage 5 Testes and penis fully grown, mature spermatozoa Regression of swelling of breasts
17–19	Increase in facial and body hair Pubic hair in Stage 6 Masculine hair border on forehead Epiphyseal fusion and growth standstill

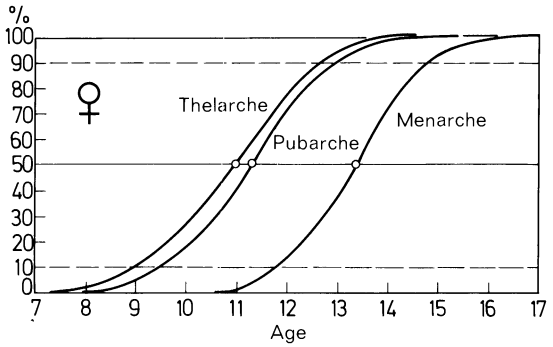


Fig. 18. Chronological scattering of the development of puberty in girls (VAN WIERINGEN, 1968)

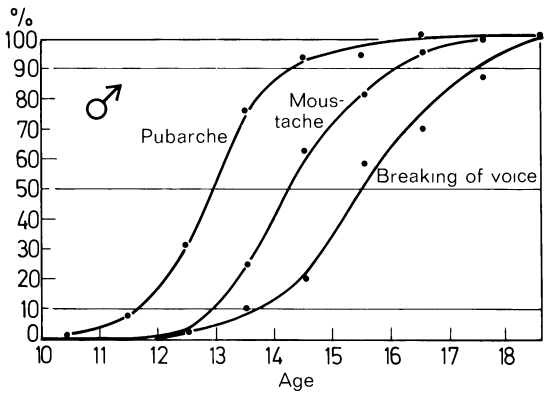


Fig. 19. Chronological scattering of the development of puberty in boys

The *striking length of the limbs* and the marked growth of the hands and feet (pubertal acromegaloid) are further features of growth during puberty. The comparative length of the extremities is greater, i.e., the ratio of upper to lower segment (p. 1024) is lower in the first phase of puberty than at any other time of life. This is explained by the fact that the extremities grow more than the trunk before and at the onset of puberty and that they also stop growing at an earlier stage. These almost eunuchoid proportions are more obvious the later puberty arises.

Sex-specific skeletal characteristics develop parallel to generalized bone maturation during puberty. These features (wide hips in the woman, broad shoulders in the man, etc.) together with the development of the musculature and adipose tissue determine the characteristic stature of the man and woman.

Development of the mammary glands or *thelarche* is the first of the *secondary sexual characteristics* to arise in the girl. The development is often unilateral to begin with, occurs more frequently on the left side, and there is often a difference between the two sides even later on. At first, the swelling of the glandular tissue can only be palpated, then the areola protudes. All stages of breast development occur during 3–4 years. At the same time, the labia minora become enlarged.

Parallel to this, gradual maturation of the vaginal mucosa can be followed in the vaginal smears and the steady increase in size of the uterus can be determined by means of rectal examination. The first pubic hair, or pubarche, appears shortly after the onset of breast development or more uncommonly before. The first sesamoid bone of the thumb becomes visible on the X-ray at about this time. This occurs at a bone age of about 11 years. The peak of the pubertal spurt of growth occurs about a year later and axillary hair develops after another year, at about the time when *menarche* arises. The first menstrual periods are usually painless, still irregular and not accompanied by the typical biphasic temperature curve (anovulatory cycle, p. 576). Fertility is not reached until 1–2 years later, with the development of regular and often painful menstruation (ovulatory cycle, p. 564).

Increase in the volume of the testes is the first *secondary sexual characteristic* in boys. Shortly after this the first pubic hair arises (pubarche) and the penis begins to grow at the same time. As in girls, the first sesamoid bone of the thumb appears on the X-ray roughly at the same time, corresponding to a bone age of 13 years. The peak of the spurt of growth during puberty is reached shortly after. Axillary hair appears later, and the beard begins to grow and the voice to break. The various characteristics become more pronounced in subsequent years. The rapid growth of the penis and testes is particularly striking, and development of these organs is complete by the age of 16. The *typical characteristics of body hair in the male* are the tapering upper border of pubic hair extending towards the umbilicus and dense generalized body hair. Beard and the temporal retrogression of the hair margin on the forehead develop later. *Swelling of the breasts* (pubertal macromastia or physiological pubertal gynecomastia) is less striking, arises in most boys at the onset of puberty, usually disappears after 2–3 years and hardly ever exceeds the diameter of the areola. It is difficult to state definitely the point at which fertility is reached, i.e. when mature spermatazoa are produced. Although pollution and ejaculation occur very early on in puberty, mature sperms are not usually formed until a few years after the onset of puberty.

Changes in the skin also belong to the secondary sexual characteristics in both sexes but particularly so in boys. Sweat excretion

increases, particularly in the axillae, and the sweat acquires a penetrating odor. Function of the sebaceous glands is greatly increased particularly in the facial region and *acne* of varying severity may be the presenting feature. The acne usually only involves the face and upper trunk and usually disappears spontaneously after a few years. *Striae distensae of the gluteal region* and occasionally also of the upper parts of the thighs is another cutaneous symptom appearing in both sexes. Little attention is paid to this symptom but it is not uncommon and does not occur only in obese children but not infrequently also in thin subjects.

Objective assessment of the size of the testes and occasionally also of the penis is very useful in the assessment of puberal disorders or hypogonadism. *Testicular volume* depends mainly on the development of the seminiferous tubules and only to a slight degree on the Leydig cells. Testes which are too small suggest inadequate spermatogenesis due to primary testicular insufficiency or gonadotropin deficiency. A conspicuously enlarged testis is often found in the absence of the contralateral testis and occasionally in the adrenogenital syndrome due to hyperplastic intratesticular adrenocortical tissue (p. 366). The simplest method for estimating the volume is comparative palpation of the testis and testicular models of known volumes (Fig. 20). The volume before puberty is about 0.75 ml to 2 ml at the most. The volume increases to 3–12 ml before the appearance of pubic hair and the final volume is reached by the 16th to 17th year (Fig. 21).

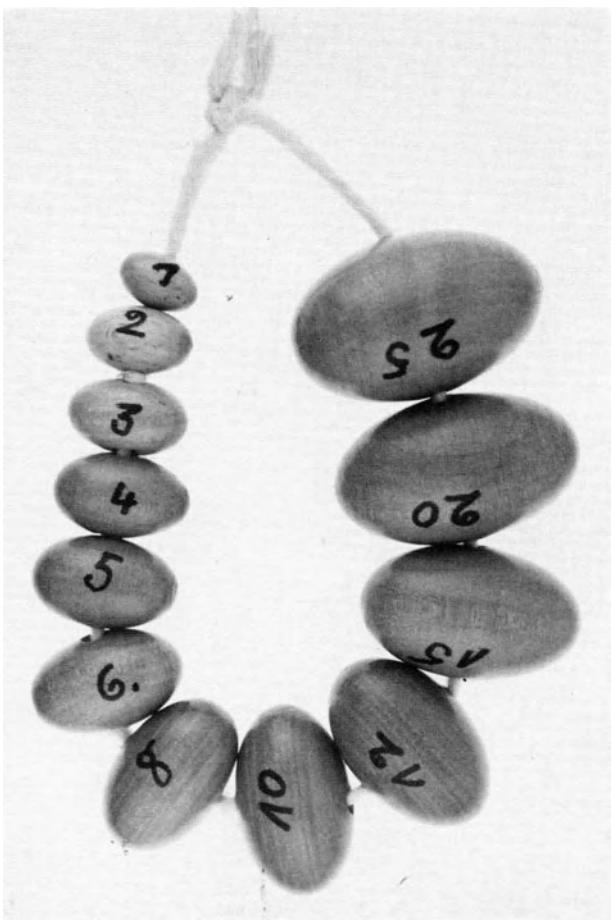


Fig. 20. Orchidometer. Models of known volumes for estimating testicular volume by comparative palpation

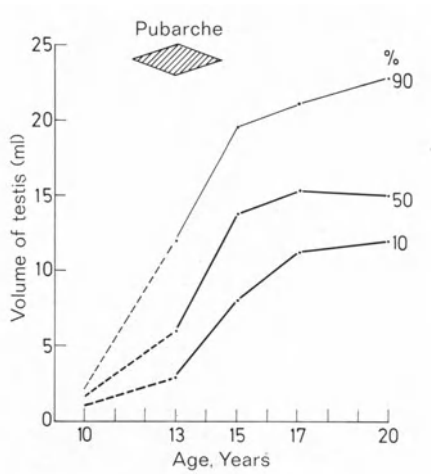


Fig. 21. Average testicular volume. Percentile 50 represents the median values. Percentile 10 (or 90) means that 10% (or 90%) of all healthy boys of the same age have smaller testes and that 90% of them (or 10%) have larger testes

The *size of the penis* is assessed by measuring the dorsal length of the stretched penis, which corresponds roughly to the length of the erect penis. The circumference of the penis may also be measured. The length of the stretched penis before puberty is about 4–8 cm and after the age of 16 it is 10–17 cm. The circumference of the flaccid penis is 3–6 cm before puberty and between 6–11 cm after the age of 16.

3. Hormonal Findings

The concentrations of gonadotropins and sexual steroids in the plasma and urine are very low before puberty, although the hypothalamo-hypophyseal-gonadal axis is definitely already in action. Shortly before secondary sexual characteristics arise or during their development the concentration of these hormones rise steadily

and reach normal adult values during or at the end of puberty.

The newly introduced radioimmunological methods for the estimation of *gonadotropins* have yielded the following picture (BAGHDASSARIAN, 1970): before puberty in both sexes LH and FSH values in the serum are between 2–6 ImU/ml (international milliunits, 2nd IRP-HMG, p. 558). These values begin to rise shortly before puberty or sometimes with the appearance of the first characteristics of puberty FSH reaching adult levels of about 5–15 ImU/ml about mid-puberty and LH at the end of puberty. Urinary values of FSH correspond to those in the plasma, rising from about 0–4 IU/24 h to 5–15 IU/24 h. LH values increase much more and reach a concentration of 20–40 IU/24 h at the end of puberty.

Urinary 17-ketosteroid levels rise slowly before puberty and very steeply during puberty (Fig. 22). At first, there is not sex difference and levels reach adult values towards the end of puberty, mean values being slightly higher in men than in women (p. 384). The rise during puberty is mainly due to the rapid increase in adrenal androgens (adrenarche) and their metabolites. This is seen in both sexes from the increase of dehydroepiandrosterone sulfate, dehydroepiandrosterone and androsterone in the plasma (MIGEON, 1957; ROSENFIELD, 1969) and of dehydroepiandrosterone, androsterone and etiocholanolone sulfates and glucuronides in the urine.

Testosterone and androstenedione are demonstrable in the plasma of children. According to FRASIER and HORTON (1966) the mean concentration of testosterone is 42 ng/100 ml in boys and 19 ng/100 ml in girls whereas the mean concentration of androstenedione is 86 ng/100 ml in boys and 30 ng/100 ml in girls. FOREST and MIGEON (1970) found a mean testosterone concentration of 25 ng/100 ml in boys and 9 ng/100 ml in girls, and a mean androstenedione concentration of 25 ng/100 ml in boys and 65 ng/100 ml in girls. They also found that the protein-bound fraction was higher than in adults. The same teams found 800 and 625 ng/100 ml testosterone and 60 and 109 ng/100 ml androstenedione in men and 42 and 49 ng/100 ml testosterone and 140 and 181 ng/100 ml androstenedione in women. It is worth mentioning that in contrast to the relative concentrations in men, androstenedione values are much higher than testosterone values in women and children and that the androstenedione concentration is lower in men than in women. From the results cited, it is even possible that the androstenedione concentration is lower in boys than in girls. This would indicate that the activity of 17-reductase (17-hydroxysteroid dehydrogenase) which is responsible for the conversion of androstenedione to testosterone, is physiologically limited in women and children. 5α-Dihydrotestosterone is found in the plasma of adults but not of children (ITO, 1970).

Testosterone is also demonstrated in urine even during childhood. Values lie below 4 μg/24 h (KNORR, 1967) and rise to normal adult levels during puberty (p. 449). In boys, testosterone production can be stimulated by means of chorionic gonadotropin even before puberty (SAEZ, 1968; RIVAROLA, 1970), the basal value

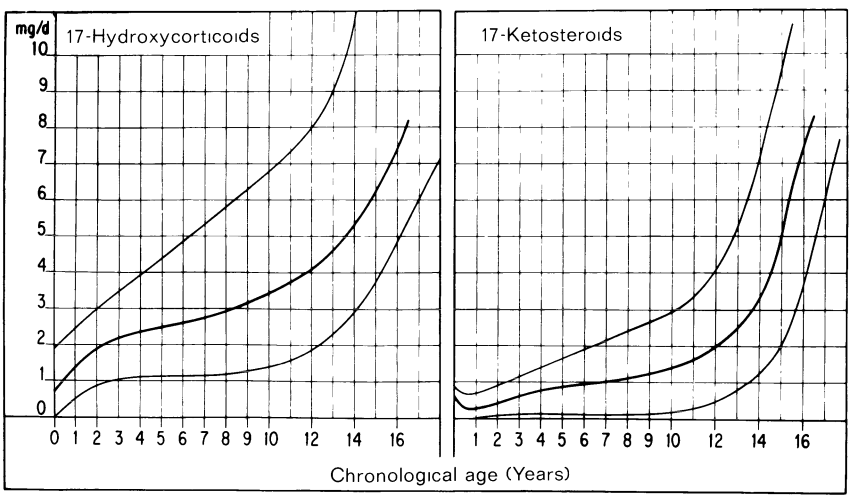


Fig. 22. Normal range of 24-hour urinary excretion of 17-hydroxycorticoids and 17-ketosteroids in boys and girls. (After KNORR, 1965).

and rise being dependent on the developmental age (ZACHMANN, 1972). It has been found that adult values can be attained after stimulation over several days.

There have been few investigations so far on *estrogens* in the plasma of children, due to technical difficulties. In urine, values between 1–3 μg/24 h have been found for estrone, between 0–2 μg/24 h for estradiol and 3–6 μg/24 h for estriol (PENNINGTON, 1969). KNORR and his team (1970) found as little as 50 ng/24 h for estrone and less than 20 ng/24 h for estradiol when they used a modern gas chromatographic method. Values rise to normal adult levels during puberty (p. 527). The same group has recently reported plasma estrogen values in children, determined by a sensitive radioimmunoassay. In males, estrone ranges from 10.6 pg/ml (pubic hair stage 1) to 31.5 pg/ml (stage 5), estradiol from 7.5 pg/ml (stage 1) to 20.7 pg/ml (stage 5). In girls, estrone values of 13.5 pg/ml (stage 1) to 74.6 pg/ml (adult women) and estradiol values from 8.2 pg/ml (stage 1) to 121 pg/ml (adult women) were found. Pregnanediol excretion is minimal before puberty. The high levels typical for the second half of the cycle are only reached 1–2 years after menarche, with the transition from anovulatory to ovulatory cycles.

In contrast to the steep rise in the production of adrenal androgens during puberty, the *production of cortisol and aldosterone* show quite a gradual increase parallel to the increase in body surface. This can be seen from the plasma concentration of cortisol, which remains constant, and from the slow rise of 17-hydroxycorticoids (Fig. 22) and aldosterone in the urine.

Basal values for growth hormone concentrations in the blood in puberty are similar to those in childhood, but after provocation with insulin and arginine, values increase above those found prior to puberty (SPERLING, 1970; FRASIER, 1970).

4. Hormonal Dependence of Secondary Sexual Characteristics

There is no doubt that the entire development of puberty is induced through the increase in androgenic and estrogenic steroids. These steroids are therefore also called sexual hormones. Whether an increased response of the target or end organs also plays a part in the appearance of pubertal characteristics has not yet been adequately investigated.

Estrogens control breast development, growth and maturation of the uterus, vagina, and labia minora, and finally menstruation. In addition, *progesterone* is also responsible for the true ovulatory cycle. *Androgens* promote growth of the penis, prostate and scrotum in boys and of the clitoris and labia majora in girls. They are also responsible for the breaking of the voice in boys and the sexual hair in both sexes.

The cause of the spurt of growth during puberty has still not been adequately explained. Both testosterone and growth hormone have anabolic growth-promoting effects. The growth hormone concentration in the blood can be raised experimentally with testosterone and estrogens after the induction of hypoglycemia with insulin. In fact, higher values are found for growth hormone in both sexes during puberty after provocation than before puberty. It therefore appears probable that androgens and estrogens work together with growth hormone to induce the pubertal spurt of growth. The more marked spurt of growth in boys can presumably be explained by the higher testosterone production.

Fusion of the epiphyses and thereby termination of growth is probably due to the action of testosterone and estrogens. Isolated growth hormone deficiency does not prevent epiphyseal fusion whereas epiphyseal fusion never occurs in the presence of a deficiency of sexual hormones.

The development of acne is connected with the increased production of androgens in both sexes. No clear reason has yet been found to explain why the acne is only temporary in spite of the continuous production of androgens. Just as little is known about the cause of the swelling of the mammary glands in boys (see also pubertal gynecomastia on p. 1041).

5. Hypothalamus, Gonadarche, Adrenarche and Puberty

It is known from experiments with animals that testosterone secreted by the fetal or neonatal testes exerts an irreversible imprinting effect on the brain (p. 707). This imprinting determines the subsequent continuous production of gonadotropins typical of the male animal. If this effect is absent due to the absence of testes and the presence of ovaries, the cyclic production of gonadotropins typical for the female later develops. It is not known whether this imprinting also occurs in the human. It appears rather doubtful, however, since female patients with the congenital adrenogenital syndrome (p. 374), who are subjected to abnormally strong androgenic influences during the prenatal

and neonatal periods, usually develop normal cycles later with adequate cortisone treatment. The question has not been definitely settled, however, since it is possible that the androgenic imprinting occurs at an earlier point and necessitates larger amounts of androgens.

The tuber cinereum is considered the hypothalamic sexual center in the human (p. 28). This center secretes gonadotropin-releasing hormones (p. 32) which stimulate gonadotropin production and hypophyseal secretion. It controls the negative feedback mechanism of the sexual hormones, either directly or indirectly via the epithalamic pineal region, i.e. it is inhibited by sexual hormones. It is probably also influenced by other central nervous factors and possibly by humoral factors as well.

The hypothalamo-pituitary-gonadal axis is already active during childhood in spite of the low concentrations of gonadotropins and sexual steroids in the blood, as has been shown in numerous animal experiments. Clinical experience is also in keeping with this, since radioimmunological methods often reveal elevated gonadotropin levels in association with Turner's syndrome even in childhood, and diminished gonadotropin values with pituitary dwarfism. In the healthy child, only very low concentrations of gonadotropins and sexual steroids acting on the negative feedback mechanism are present. This means that the hypothalamus can react even to very small amounts of sexual steroids, i.e., it is extremely sensitive (DONOVAN, 1965). This sensitivity disappears during puberty. Because of this, more gonadotropins are secreted and this in turn causes an increased production of sexual steroids in the gonads. The activity of the hypothalamo-pituitary-gonadal axis is thus intensified to give a higher concentration of gonadotropins and sexual steroids.

Clomiphene (p. 599) administered to adults in a dosage of 25–200 mg causes gonadotropin secretion to be increased (positive feedback mechanism) whereas in a dosage of 500 mg it inhibits gonadotropin secretion (negative feedback mechanism). A dose of 1–100 mg/m^2 before puberty has an inhibitory effect on plasma gonadotropins whereas a smaller dose has no effect (KULIN, 1969, 1970). The known competitive action of estrogens and clomiphene on the hypothalamus causes the positive feedback mechanism in adults, whereas the mild estrogen-like action of clomiphene explains the negative feedback mechanism. The negative feedback mechanism, which can be induced by much smaller doses before puberty, is indirect proof of the increased sensitivity of the hypothalamus to sexual steroids during this phase of development. This does not adequately explain the absence of a positive feedback mechanism before puberty, however.

The factors inducing puberty, i.e. the factors causing the decrease in sensitivity of the hypothalamus to sexual steroids, are not known. It is possible that inhibitory central nervous influences from the pineal region or other centers disappear or that gonadotropic-inhibiting humoral factors (SOFFER, 1965) characteristic of childhood also vanish. But, even with these assumptions another cause must be searched for, since they do not explain why the development of puberty is associated less with a certain age or a certain body height and much more with a certain body maturation, which is best assessed from bone age. More recently, body weight has also been looked upon as a possible standard factor for the onset of puberty (FRISCH, 1970).

Gonadal maturation induced by the increased gonadotropin concentration and the associated increase in the production of sexual hormones is also known as *gonadarche*. The adrenals show a similar maturation process during puberty, which is called the *adrenarche*, although only androgenic production is affected and production of cortisol and aldosterone is not. This is illustrated clinically by the fact that adrenal failure leads to loss of pubic hair in women whereas gondal failure has no such effect. Adrenal androgens appear to be responsible for the development of pubic and axillary hair. Adrenarche is also manifest in the hormonal findings in the blood, the concentration of dehydroepiandrosterone sulfate in the blood rises rapidly during puberty (ROSENFIELD, 1969).

It has been shown that gonadarche is controlled by the gonadotropins whereas the regulation of adrenarche remains unsolved. ACTH need hardly be considered since it would also cause a steep rise in cortisol production during puberty, which does not in fact occur. The possibility of a stimulant action of LH on the androgen production of the adrenals is disputed and largely rejected. It is possible that adrenarche is induced and controlled by some pituitary hormone which is still unknown. Certain observations suggest that adrenarche is stimulated directly or via this hypothetical pituitary hormone by the sexual hormones produced by the gonads. Thus, the very poorly developed pubic hair in an untreated Turner's syndrome usually improves with estrogen treatment whereas the same treatment in cases of anterior pituitary deficiency does not promote growth of pubic hair. Furthermore clomiphene, which has a slight estrogenic action (p. 599), has been found to induce higher plasma levels of andro-

stenedione and dehydroepiandrosterone sulfate when given before puberty (CATHRO, 1969).

The physical development during puberty can be said in summary to be due to the combined action of gonadarche and adrenarche. In contrast to this, cases of isolated premature pubarche are probably due to premature adrenarche in the absence of gonadarche while cases of precocious puberty are probably caused by isolated premature gonadarche without adrenarche.

F. Special Variations of Normal Pubertal Development

1. Survey

As mentioned above, the time of onset of puberty and the order in which the pubertal characteristics develop and the degree of development of the individual features are very variable. Wide deviations from the mean should not be considered pathologic providing the object of pubertal development is reached – i.e. maturation to healthy adulthood and the capacity for procreation. Conspicuous deviations usually have to be recorded as constitutional or idiopathic variations of normal pubertal development as the ultimate reasons for them are unknown. The conditions listed below belong to this group.

1. Puberty arising unusually early or late but otherwise normal: *idiopathic precocious puberty* (p. 1045) and *idiopathic delayed puberty* (p. 1054).

2. Isolated premature appearance of individual secondary sexual characteristics: *premature pubarche* (p. 1040) and *premature thelarche* (p. 1041), which are both quite common, and *premature menarche* which is very rare.

3. Severe and persistent *pubertal gynecomastia* (p. 1041).

4. Generalized somatic, psychic and psychosexual *variations of "masculinity" and "feminity"* (p. 1042) which are completely unrelated to true intersexuality.

5. Obesity and emaciation arising before or during puberty: prepubertal and pubertal obesity (p. 1043) and pubertal emaciation (p. 1045).

Idiopathic forms of precocious puberty and delayed puberty are discussed with pathological forms in a different section (p. 1045) and the other observations and syndromes are dealt with below.

2. Isolated Premature Pubarche

Isolated premature pubarche, i.e. premature appearance of sexual hair without other secondary sexual characteristics, is seen more often in girls than in boys, and particularly frequently in children with cerebral damage (cerebral palsy, idiocy, epilepsy). Hair develops during babyhood or infancy, is limited at first to the labia majora (Fig. 23) or root of the penis and then extends very slowly into the pubic region. Axillary hair appears after a longer interval than in normal puberty. This condition differs from precocious puberty in that all the other secondary sexual characteristics are absent, and from the adrenogenital syndrome in the absence of any signs of virilization. Careful examination reveals only a slight advance in growth and bone maturation. The course is completely benign and true puberty develops at the normal time with the appearance of the remaining secondary sexual characteristics.

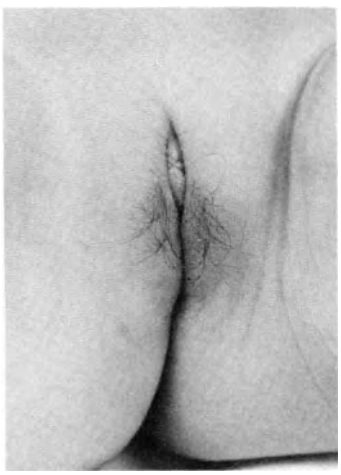


Fig. 23. Premature pubarche in a girl of 4 years (KspZ)

The excretion of 17-ketosteroids is slightly elevated. This is due mainly to a significant rise in the levels of dehydroepiandrosterone, androsterone and etiocholanolone, which are derived predominantly from adrenal androgens (VISSER, 1966). In contrast to the adrenogenital syndrome, testosterone is normal or only slightly elevated. The same steroids have been found to be raised and to sink to normal levels with dexamethasone in isolated cases (CONLY, 1967). Serum levels of LH and FSH are normal (PENNY, 1970) or even slightly diminished (KENNY, 1969). This may be attributable to the inhibition of gonadotropins by the adrenal androgens, working via the hypothalamus, which is very sensitive before puberty (p. 1039).

This condition is obviously due to a premature rise in the androgen production (premature adrenarche). In contrast to normal puberty and to true precocious puberty, there is no simultaneous gonadal activation. Associated brain damage is frequently found, which indicates a hypothalamic cause, but it is still not clear what mechanisms are responsible for the premature induction of adrenarche.

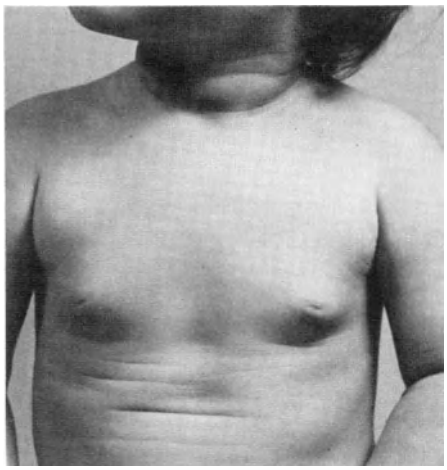


Fig. 24. Premature thelarche in a 2-year-old girl (KspZ)

3. Isolated Premature Thelarche

It is not particularly uncommon to find swelling of the mammary glands in otherwise normal girls during babyhood and infancy. The size of the swelling usually varies between that of a hazel nut and that of a plum, but there is no enlargement or pigmentation of the nipples or areolae and there are no other signs of sexual precocity, i.e., no pubic hair and no enlargement of the labia minora and uterus (Fig. 24). Vaginal smears and the cellular sediment of the urine often demonstrate a mild estrogenic action. Growth, bone age and excretion of 17-ketosteroids are appropriate to chronological age. The swelling may last for a few months or even years, after which it regresses spontaneously or gradually gives way to normal puberal development. The cause is probably a trivial premature production of estrogens by the ovaries (premature gonadarche). Serum levels of LH and FSH are normal (PENNY, 1970) or slightly raised (KENNY, 1969).

4. Puberal Gynecomastia

In a few boys the physiological mammary swelling or macromastia during puberty reaches such proportions that it can hardly be distinguished from normal female mammary development. This form of gynecomastia is usually bilateral and more rarely unilateral, more often on the left side. Occasionally it appears to be hereditary. In a few isolated cases, the breasts may become as large as those of a young woman. Whereas physiological swelling of the breasts during puberty disappears after a few months or years, this form usually persists unchanged into adulthood. Milk secretion does not occur.

The mammary tissue does not differ histologically from that in other forms of gynecomastia (p. 487).

Apart from the gynecomastia, there are no pathologic findings in the somatic development. The testes are of normal size, secondary sexual characteristics are normally developed and psychosexual development is normal. Shame and fear of being made fun of by their associates, and the endogenous uneasiness about the de-

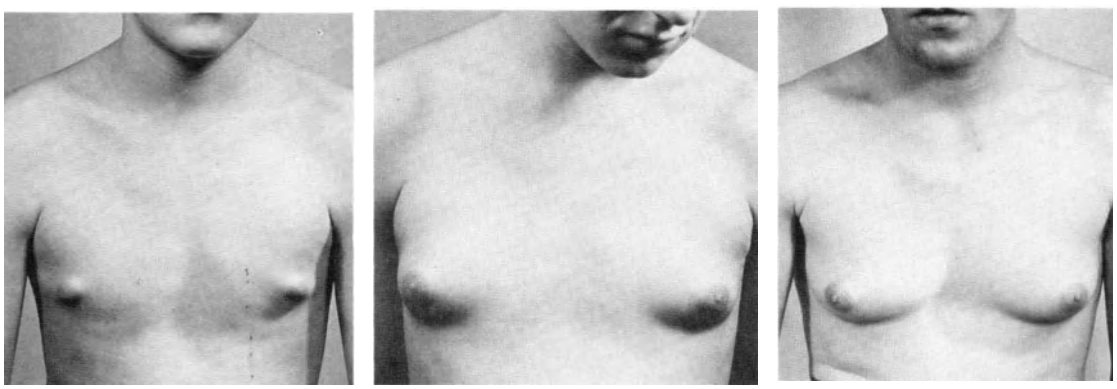


Fig. 25. Puberal gynecomastia of varying degrees in 3 boys with otherwise absolutely normal puberal development, testes of normal size and normal gonadotropin and 17-ketosteroid excretion (PRADER, 1955)

velopment of the breasts, cause these boys to hide their "trouble" and make a secret of it, avoiding gymnastics and swimming and withdrawing more and more from their surroundings.

The cause of this syndrome is unknown. Excretion of 17-ketosteroids, estrogens and gonadotropins does not appear to deviate significantly from normal, although a mild estrogenic action is found in the cellular urinary sediment. Increased estrogenic production by the testes and an age-specific metabolism of testosterone (liver) may be involved.

The diagnosis is usually easy but other causes of gynecomastia (p. 488) must always be considered as well. Apart from this, gynecomastia can also be simulated by severe panniculus (pseudogynecomastia). Differentiation from the gynecomastia in *Klinefelter's syndrome* is extremely important. Secondary sexual characteristics are more or less normally developed in both syndromes. Testicular size is the only clinical difference. In *Klinefelter's syndrome* the testes are small whereas they are of normal size in puberal gynecomastia. In doubtful cases, assessment of the sex chromatin, measurement of the gonadotropins in the plasma and urine, investigation of the sperms, and testicular biopsies will allow differentiation. These investigations all yield normal results in puberal gynecomastia, in contrast to *Klinefelter's syndrome*. Differentiation between the two syndromes is important since puberal gynecomastia is only a constitutional variation with normal reproductive function whereas *Klinefelter's syndrome* is a true endocrinopathy associated with sterility. Idiopathic gynecomastia in boys of prepuberal age is very rare but must also be differentiated from puberal gynecomastia.

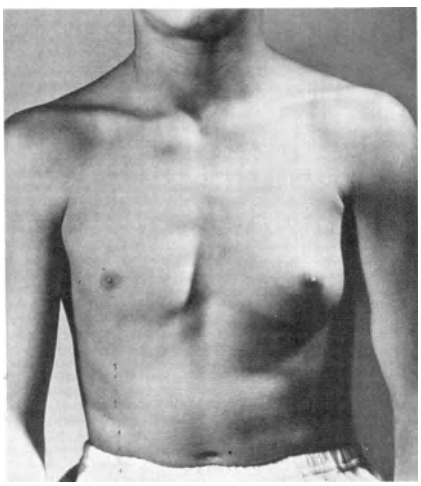


Fig. 26. Very marked unilateral puberal gynecomastia in a 15-year-old boy (KspZ)

There is no hormonal *treatment* for puberal gynecomastia. Administration of chorionic gonadotropin or testosterone is useless and illogical since these drugs themselves occasionally induce gynecomastia. In addition, such treatment can only inhibit endogenous pituitary and testicular function. The doctor must explain the harmlessness of the disorder to the patient and his relatives and warn them against hormonal treatment. If the breasts show no tendency to regress spontaneously surgical removal is the only possibility left. This decision is reached earlier when the gynecomastia produces severe psychological reactions. The incision is made along the edge of the areola and extended outward to the side. The cosmetic results of this incision are excellent.

5. Generalized Somatic, Psychic and Psychosexual Variations of "Masculinity" and "Femininity"

Only a very small proportion of the numerous physical and psychological differences between healthy individuals can be explained by hormonal variations. Most of these differences are due to constitutional and exogenous factors independent of the hormonal interaction. This must be borne in mind particularly during puberty. "*Feminine*" *features* become obvious *in individual boys* shortly before and at the onset of puberty. The body is rounded, the pelvis broad, the shoulders narrow, the voice soft and clear, and body movements girlish and affected. Conversely *girls* with "*sporty masculine*" *features* are also seen, with broad shoulders, narrow hips, dense body hair (hirsutism, p. 359), well-developed musculature, harsh voice, and energetic movements. Psychological features reminiscent of the other sex are often found in the same individuals. The boys are sensitive, shy, blush easily, like to stay at home, read a lot and avoid sports and company. Conversely, some girls are loud and noisy, energetic and robust. They are not interested in clothes and books but in sports and outdoor games.

This *combination of masculine and feminine features in stature and psyche* often causes relatives to suspect a "glandular disturbance" and to fear sexual abnormality or sterility, especially in cases where boys are obese and puberty is delayed. Thorough physical examination, however, hardly ever reveals any actual proof of an endocrine disorder. One of the doctor's main duties is to speak to the parents of these children and to talk them out of their ideas of "glandular disorders" and "abnormal

sexual tendencies". It is never admissible to deduce somatic intersexuality or homosexuality from the symptoms mentioned. Some or most of these features disappear during puberty and these children often develop into perfectly ordinary adults.

Relatives occasionally find another source of worry in the undirected sexuality evolving during puberty, which is particularly strongly developed in boys and leads to renewed suspicions of "glandular disorders" or "abnormal sexual inclinations". Reasons precipitating these fears are close friendship with members of the same sex, masturbation, and isolated homosexual acts. All these features belong to the *physiological, psychological and sexual unrest and urgency of adolescence* and spontaneously give way to unobtrusive mature heterosexual sexuality. The best counter to it all is a truthful explanation of the facts of life at an early stage and a healthy, honest relationship between parents and child, with the opportunity of discussing sexual problems openly. Again, the doctor must correct parental fears; he should remind the parents of their own youth and plead for understanding in these puberal crises. A talk with the youth, making him able to feel that the doctor is well disposed towards him and understands his difficulties, is the best form of psychotherapy and usually permits the doctor to recognize the isolated cases needing psychiatric treatment.

6. Prepuberal and Puberal Obesity

Although obesity at any age presents a difficult medical problem, an underlying endocrine disorder is never so frequently suspected as with prepuberal and puberal obesity. In many cases these children were abnormally fat during infancy, but the obesity often starts after the 8th to 10th year and reaches its peak shortly before puberty or at the onset of puberty. In addition, the panniculus undergoes most of its physiological increase during these years, and children of this age tend to put on weight particularly easily.

The panniculus is usually uniformly distributed. The skin is usually normal but may often be strikingly red. In severe cases there may be pale or red striae distensae on the hips, thighs, and buttocks, and laterally on the breasts. In the majority of cases, growth is slightly in advance of the average for the age and the skeleton is broad and powerfully built. Hands and feet are often strikingly small in comparison (aeromicria) but acromegaloid features of puberty are also often dominant. Knock knees and foot deformities are frequently present and can be explained as static damage due to the obesity. Blood pressure is normal or slightly elevated. These patients are usually languid, good natured, and passive. A good case history usually reveals that nutrition is very ample or excessive.

Puberty usually arises prematurely in *girls* (Fig. 27), in keeping with the accelerated bone maturation. Growth also ceases earlier and occasionally even before normal adult height is reached. The obesity, very wide hips and the often massively developed breasts, consisting of glandular tissue and panniculus, often give these girls a matronly appearance when they are still quite young. In *boys* (Fig. 28), the onset of puberty tends to be later than average but still within the normal age range. The mild gigantism, the slight delay in puberty, the resultant mildly eunuchoid proportions (p. 1022) and the fact that the genitalia are almost completely obscured by panniculus suggest hypogonadism to the inexperienced. This impression is often supported by the isolated feminine features such as soft rounded body curves, simulated breast enlargement, soft, shy voice and girlish movements. Parents are struck by all these symptoms, suspect a glandular disorder, and consult the doctor, whose main problem is then to decide whether the boy has *Fröhlich's syndrome* (dystrophia adiposogenitalis).

The case described by Fröhlich was that of a boy in whom a hypothalamic tumor led to mild obesity, permanent hypogonadism and inhibition of growth. Although the syndrome described, later also termed dystrophia adiposogenitalis, was exactly defined and included the hypothalamic disorder, some authors subsequently applied this term to all boys with obesity and infantile genitalia, i.e., all fat boys not yet in puberty.

When prepuberal and puberal obesity are termed dystrophia adiposogenitalis, these boys are branded with the suspicion of hypogonadism and subjected to incorrect and unnecessary hormone treatment when no hypogonadism or endocrinopathy is in fact present. Thorough examination always shows that the genitalia only appear to be infantile because of the padding of pubic fat and that the scrotum, penis, and testes are in fact normally developed for the age.

Metabolic investigations often show that there is a marked rise in insulin levels during the glucose tolerance test and that glucose tolerance is occasionally reduced at the same time. It is also found that the sensitivity to insulin is reduced and there is no rise in the growth hormone concentration in the blood

after stimulation with insulin or arginine. The basal metabolic rate, related to body surface or size of the patient, is usually within normal limits. Steroid excretion is normal; corticoid excretion may be slightly raised but not high enough for the diagnosis of Cushing's syndrome.

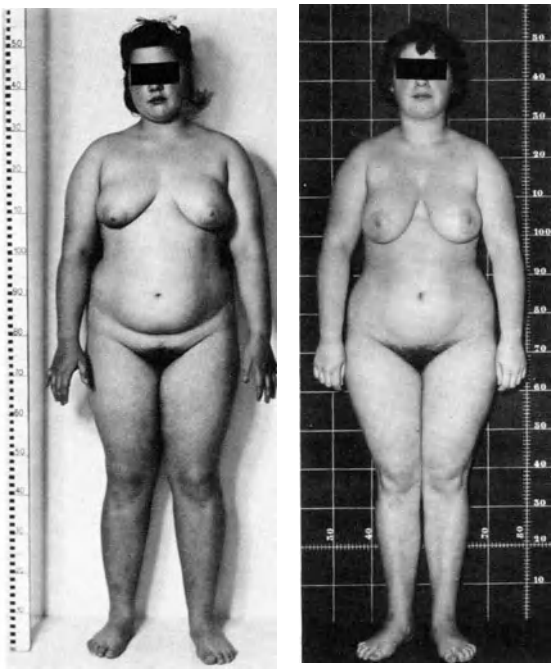


Fig. 27. Two 13-year-old girls with puberal obesity. Note the marked breast development and matronly stature which make these girls look older (KspZ)

There is no doubt that the main *cause* is *the high nutritional intake*, supported to some extent by the *eating habits* of the family. Too little physical exercise and often an unfavorable mother-child relationship also play a part; the mother often fusses over the child and protects him in an exaggerated manner (overprotective mother), gives him too much care and too much food, pampers and watches over him during school age as if he were still an infant, prevents normal relationships with other children and thus makes healthy psychological development impossible. This domineering attitude of the mother is interpreted as compensation for the neglect she herself experienced as a child or as a subconscious rejection of the child. As a result, these children become emotionally embittered, are dissatisfied, sullen and bored and find satisfaction only in eating. In addition the family seems to be predisposed to obesity, but it is difficult to distinguish between true heredity and acquired eating habits. The involvement of hormonal factors in the *pathogenesis* is still a controversial subject. In any case there is no definite proof of this.

No definite *prognosis* can be made. A few patients lose weight towards the end of puberty (Fig. 28) but this is probably due to the reduction of food intake resulting from a changed psychological situation. In the majority of patients, however, the obesity persists unchanged into adulthood.

In the *differential diagnosis*, the more uncommon forms of obesity associated with hypothalamic disorders, Cushing's syndrome (p. 340), and hypothyroidism (p. 165) must be considered. In addition to the postencephalitic and posttraumatic forms of the true Fröhlich's syndrome, pituitary dwarfism and the Prader-Labhart-Willi syndrome (p. 810) belong to the group of organic hypothalamic disorders. The Laurence-Moon-Bardet-Biedl syndrome is transmitted on an autosomal recessive gene and is characterized by debility, retinitis pigmentosa, polydactyly and moderate obesity. Dwarfism and hypogonadism are occasionally also present.

All these organic forms of obesity occur much less frequently and can be differentiated from most cases of prepuberal and puberal

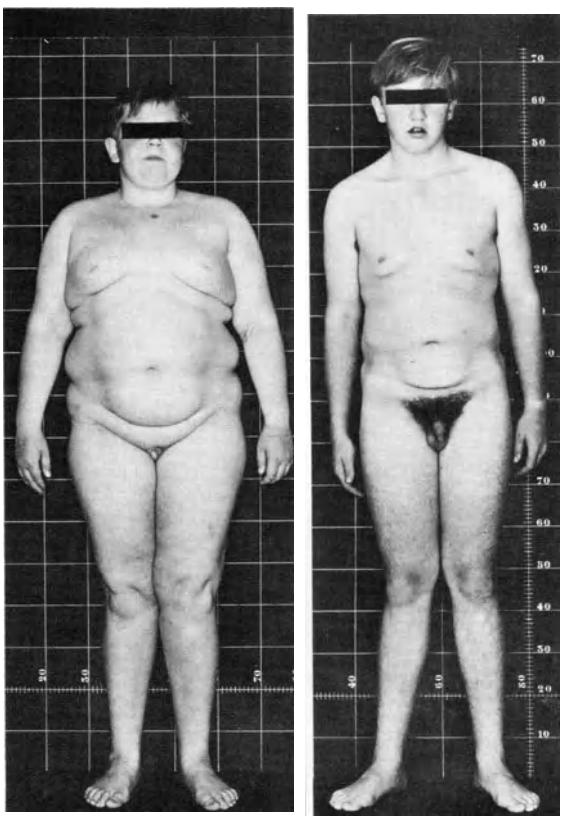


Fig. 28. Endocrinologically healthy boy with psychogenic prepuberal obesity at the age of 13 years (left), and after slimming at the age of 15 (right) with absolutely normal puberal development

obesity as dwarfism is almost always present in organic forms. The following general rule can be applied to children up to the age of puberty: there is usually no endocrinopathy and no organic cause whatsoever for obesity combined with above-average height, whereas careful seting for the endocrinopathies listed above and hypothalamic disorders is indicated in cases of obesity with dwarfism.

As in other benign deviations from normal puberal development, *therapeutic* measures consist in convincing the parents and patients that there is no "glandular disorder" by pointing out any puberal factors already apparent or by indicating the favorable prognosis of the onset of puberty, which can be precisely predicted chronologically from bone age. The obesity must be treated by diet and increased physical exercise. Amphetamines should not be used to inhibit the appetite in this age group or only with extreme caution. Special psychotherapeutic attention is advisable in isolated cases. Treatment with pituitary or thyroid extracts or with sexual hromones has no scientific basis and must be rejected as a pointless intervention in the endogenous hormonal equilibrium.

7. Puberal Emaciation

Puberty is the commonest time for the onset of psychogenic anorexia mentalis or anorexia nervosa in the female sex. The extreme emaciation was previously looked upon as a result of a pituitary insufficiency, but since the picture of pituitary insufficiency in women has become familiar (p. 93), puberal emaciation is hardly ever still mistaken for endocrine disorders. As in other severe generalized illnesses, there is often a secondary reversible failure of gonadotropins and sexual steroids, and growth often ceases while basal growth hormone values are normal or increased but in many cases cannot be stimulated by insulin or arginine. The other endocrine functions are completely or almost intact (p. 1028).

G. Precocious Puberty

There is no clear biologically based definition of premature puberty or precocious puberty. In general precocious puberty is the term used when puberal characteristics arise before the age of 6 in girls and before the age of 8 in boys. "Premature puberty" is used when signs of puberty arise between the 6th and 8th years in girls and between the 8th and 10th years in boys.

1. Survey

a) Etiological Classification of True Precocious Puberty and Precocious Pseudopuberty

True hypothalamic precocious puberty with the premature development of puberty is due to a premature secretion of hypothalamic gonadotropin-releasing factors. These stimulate the production of gonadotropins by the pituitary, which is followed in turn by gonadal maturation. Gonadal function is no different from that reached during normal puberty. The ability to reproduce is also reached during premature puberty just as during normal puberty.

Premature puberty frequently occurs with tumors and other disorders affecting the hypothalamus, but there is no known pituitary tumor associated with precocious puberty. In contrast to hypothalamic precocious puberty, there is no primary pituitary precocious puberty.

If the symptoms of premature puberty are not induced by premature hypothalamic activity, the term *pseudosexual precocity* is used. The hormones responsible for the signs of puberty are derived from tumors or diseased glands or obtained from an exogenous source. Neither hormone production nor gonadal function is as in normal puberty. Patients are therefore also infertile. This classification into pseudo- and true precocity does pose some problems, since fertility is theoretically possible in cases with gonadotropin-producing tumors and interpretation as either pseudo- or true sexual precocity would be possible in such cases.

The different forms of precocious puberty in boys and girls are summarized in Tables 11 and 12. True idiopathic precocious puberty in girls is by far the most common form, followed by pseudosexual precocity affecting both sexes equally and associated with the adrenogenital syndrome.

b) Features Common to all Forms of Precocious Puberty

The *secondary sexual characteristics* are the most striking features in all forms, can arise at any age, and may even be present at birth. The order in which the individual secondary sexual characteristics arise and the time interval between them vary very much more widely than in normal puberty. The rapid appearance of all the secondary sexual characteristics suggests a brain or gonadal tumor or a gonadotropin-producing tumor. In addition, growth and bone development are found to be accelerated, i.e. *general somatic development is advanced.* The extent of this advance is usually proportional to the length of history of the sexual

Table 11. Precocious puberty in boys

	Hormonal findings		Size of testes	Testicular histology T = tubules + spermiogenesis L = Leydig cells
	Increased gonadotropins	Increased ketosteroids		
1. *Hypothalamic precocious puberty*				
a) Idiopathic, constitutional b) Organic brain disorders c) Hormonal overlapping	+	+	Small to definitely enlarged	T immature to mature L +
2. *Gonadotropin-producing tumors*				
a) Chorionepithelioma, teratoma	+ + +	+/+ +	Small to slightly	T mainly immature
b) Hepatoma	+ + +	+/+ +	enlarged	L +
3. *Sexual hormone-producing tumors or hyperplasias*				
a) Adrenal cortex (adrenogenital syndrome)	0	+ + +	Small or enlarged	T immature, L 0
b) Testes (Leydig cell tumor)	0	+ + +	Unilateral tumor-like enlargement	T immature, L 0
4. *Intake of exogenous hormone*				
a) Chorionicgonadotropin	+	+ +	Slightly enlarged	T immature, L +
b) Androgens and anabolic steroids	0	+/+ +	Small	T immature, L 0
c) Estrogens	0	+	Small	T immature, L 0

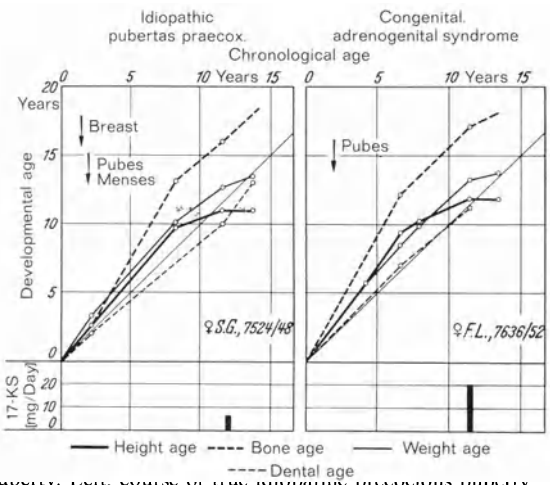
Table 12. Precocious puberty in girls

	Hormonal findings			Mammae, genital development, menses	Pubic hair
	Increased gonadotropins	Increased 17-ketosteroids	Increased estrogens		
1. *Hypothalamic precocious puberty*					
a) Idiopathic, constitutional	+	+	+	+	+
b) Organic brain disorders	+	+	+	+	+
c) Fibrous bone dysplasia	+	+	+	+	+
d) Hormonal overlapping	+	+	+	+	+
2. *Gonadotropin-producing tumors*					
Chorionepithelioma, teratoma	+ + +	+/+ +	+/+ +	+	+
3. *Sexual hormone-producing tumors or hyperplasias*					
a) Adrenal cortex (adrenogenital syndrome)	0	+ + +	+	0	+
b) Ovaries (granulosa cell tumors etc.)	0	+/+ +	+ +	+	+
4. *Intake of exogenous hormones*					
a) Estrogens	0	+/+ +	+ +	+	0/+
b) Anabolic steroids	0	+/+ +	0/+	0	+

precocity, and occasionally it is absent when the history is very short. In contrast to normal puberty, bone age at the beginning of precocious puberty has not reached the stage usual at puberty in spite of acceleration of bone development. Cases with harmonic development of puberty and bone age must be considered as early normal puberal development and not as a pathologic form of puberty. Accelerated bone development results in premature epiphyseal fusion and cessation of growth before normal adult height is reached. In other words, the patient is taller than normal as a child and shorter than normal as an adult. Nevertheless, many of these patients reach an adult height within the lower normal limits. This entire process is illustrated by two examples in Fig. 29.

In contrast to the general advance in somatic development, psychological, psychosexual and intellectual development usually correspond to chronological age. Exceptions with advanced psychological and intellectual development and cases of debility (brain disorders) are, however, not very uncommon. The discrepancy between

somatic and psychological development is usually so marked that difficulties arise at home and at school. The advanced somatic development arouses the penetrating curiosity of other children and also, unfortunately, of adults and causes too high psychological and intellectual demands to be made. Patients suffer less from the premature puberty than from the psychoreactive disturbances caused by the striking external changes and from the unskilled psychological attitude of other people. The children are therefore often confused, shy, and withdrawn, but often become coarse and aggressive as soon as they become aware of their superior strength. Early sexual interest and aggression are much less common than would be expected from the anecdotes in ancient literature. The disprepancy between the childish psyche and the somatic sexual maturity becomes particularly grossly apparent when girls with precocious puberty become pregnant and give birth to children. According to most observers, these young mothers have no understanding of the significance of pregnancy and birth It is the doctor's duty to help the parents to understand these children and to assist with the difficult problem of education.



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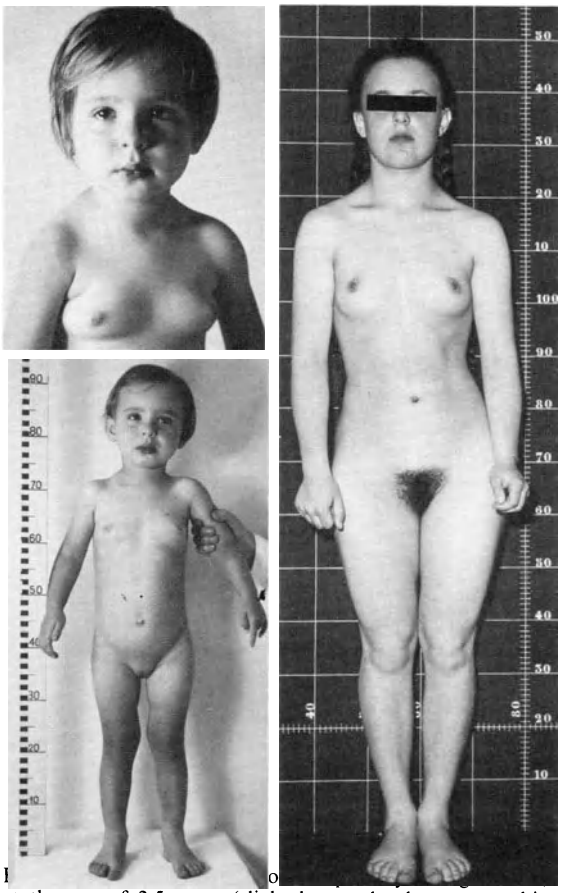
puberty. Left, course of true idiopathic precocious puberty in a girl (same patient as in Fig. 30). Right course of pseudosexual precocity due to a congenital adrenogenital syndrome in a girl. Note the acceleration of growth and bone development, premature standstill of growth, and normal dental development. This course is typical for all forms of premature puberal development (KspZ)



I
at the age of 2.5 years (slight breast development, pubic hair not marked, menarche). Right, at the age of 13 years 2 months, growth already terminated (epiphyseal fusion). The growth curve and remaining developmental data of this girl are presented in Fig. 29 (left) (KspZ)

2. Idiopathic Precocious Puberty

Idiopathic, constitutional or genuine precocious puberty is by far the most common form of premature puberty (Fig. 30). Girls are affected 4 to 7 times more frequently than boys. Most cases in girls are sporadic. Familial cases have been described, especially in boys, whereby the condition can be inherited via healthy mothers as well as via affected (occasionally also healthy) fathers. The mode of inheritance is presumably autosomal sex-lined dominant. Families with autosomal recessive inheritance in which sexual precocity arises not only in boys but also in girls are much less common.

Most affected children have a normal, and often shortened, puberty, but puberty is sometimes very prolonged, and spontaneous regressions occasionally occur (transitory precocious puberty). The children are extremely healthy; emotional and intellectual development correspond to age, and life expectancy is normal. Reproductive function is normal. Pregnancy in little girls with idiopathic precocious puberty has frequently been reported. The youngest reported case, who gave birth to a child by Cesarean section, was 5.5 years old (ESCOMED, 1939).

The expected gonadotropin excretion was often not detected in investigations in the past. With more modern methods and repeated investigations, however, it has been found that values of LH and FSH in serum and urine are usually just as high as in puberty arising at the normal time. The 17-ketosteroid excretion is only slightly higher than normal values, and is lower than during normal puberty. In contrast to this, the excretion of estrogens in girls and of testosterone in boys is significantly elevated and often as high as normal adult values. The combination of low levels of 17-ketosteroids and high levels of sexual steroids in the urine indicates that in contrast to the situation during puberty arising at the normal time, only gonadal steroid production (gonadarche), and not adrenal androgen production (adrenarche), has started to function prematurely. In other cases, however, 17-ketosteroid levels are found to be as high as those in normal puberty. The diagnosis of idiopathic precocious puberty should only be made after all other forms of sexual precocity have been excluded as far as possible. Periodic neurologic examination is essential because of the possibility of a clinically silent brain tumor. Treatment is discussed on p. 1053.

3. Precocious Puberty with Organic Brain Disorders

Precocious puberty due to organic brain disorders (*cerebral or neurogenic precocious puberty*) is quite uncommon. It is seen with hyperplastic malformations of the tuber cinereum (hamartoma) and also with various cerebral disorders such as malformations of the brain, hydrocephalus (Fig. 31), postencephalitic states, brain tumors, von Recklinghausen's neurofibromatosis, tuberous brain sclerosis and occasionally also with mongolism.

The hamartoma of the tuber cinereum is a hyperplastic malformation of the sexual center. It presumably produces gonadotropin releasing factors autonomously, thus leading to the premature development of puberty. Since it is a very small structure, there are often no general symptoms of a brain tumor.

The other cerebral lesions giving rise to precocious puberty are usually accompanied by *hydrocephalus of the third ventricle* and by nonspecific changes of the tuber cinereum, so that these forms of precocious puberty are probably also induced via the sexual center in the tuber cinereum. Definite neurologic disturbances are usually found during the general examination.

Tumors of the pineal body are a special form of localized tumors which arise almost exclusively in boys and often give rise to precocious puberty (pineal precocious puberty). Calcification of these tumors is frequently visible radiologically. The pathogenesis of precocious puberty with tumors of the pineal body is just as controversial as the endocrine function of the pineal body. If the pineal body normally inhibits puberal development (p. 1039), the precocious puberty is due to a loss of his inhibition. If the pineal body normally has no endocrine function, it is more likely that these tumors induce premature puberty via a direct or indirect pressure effect on the sexual center in the tuber cinereum. Unfortunately, pathological investigations have failed to solve this problem.

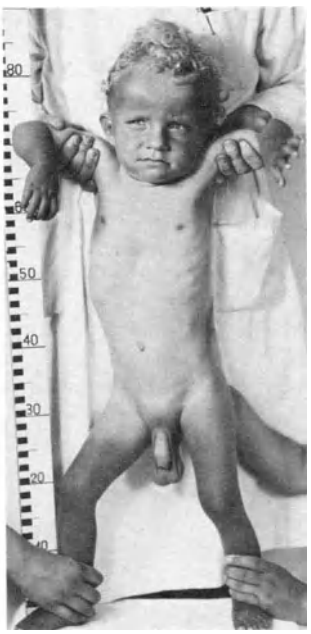


Fig. 31. True precocious puberty due to hydrocephalus (brain tumor?) in a boy aged 1 years 10 months (KspZ)

Apart from the fact that pregnancies have never been observed in neurogenic precocious puberty, the hormonal situation in neurogenic precocious puberty is no different from that in idiopathic precocious puberty.

The decisive diagnostic findings are neurologic in nature. Often, gross neurologic disturbances are present, such as epilepsy, manifest hydrocephalus, cerebral palsy, or severe idiocy, and these indicate a cerebral cause of the precocious puberty at once. Special attention must be paid to signs of increased intracranial pressure (headaches, vomiting, papilledema), calcifications in the region of the pineal body (tumors of the pineal body), general diencephalic metabolic symptoms (polyuria, polyphagia, nycturia,

obesity), and ocular disturbances (visual disturbances, limitation of visual fields, paralysis of ocular muscles). Conjugate paralysis on looking upwards is a very important symptom in certain tumors of the mid-brain (Parinaud syndrome).

4. Precocious Puberty with Fibrous Dysplasia of the Bones

This form of precocious puberty, which is also known as Albright's syndrome or the McCune-Albright syndrome (McCune, 1937; Albright, 1937), occurs almost exclusively in the female sex and is characterized by the following triad of symptoms: 1. true precocious puberty, 2. polyostotic fibrous dysplasia (osteodystrophia fibrosa disseminata) and 3. map-like, milk-coffee colored skin pigmentations. A goiter is often present and hyperthyroidism has occasionally also been observed.

Bone changes are clinically silent or become apparent mainly because of asymmetric bone deformities (shepherd's staff shape of the femora in particular), spontaneous fractures or distension of the facial bones. X-rays show cystic translucent areas in the long bones, arranged unevenly and often involving only one half of the body, osteosclerosis and hyperosteosis in the region of the base of the skull, orbits, and facial bones. The bone changes are painless. Calcium and phosphorus metabolism appears to be undisturbed. Serum levels of alkaline phosphatase are only slightly elevated at the most. The histological picture consists of tough, connective tissue containing few cells, which replaces normal bone tissue. The same disorder occurs in both sexes, often in the absence of endocrine symptoms and with or without skin pigmentation (osteodystrophia fibrosa disseminata, Jaffé-Lichtenstein).

The patches of *pigmentation* vary very widely in size and localization. They are not raised, are the color of milk coffee, and have indistinct borders. They show a predilection for the skin segments corresponding to the bone lesions and are often localized to the same half of the body as the bone lesions.

The *etiology* of this strange syndrome is not known, but the condition is probably due to a combination of disorders. Most authors seem to think that this form of precocious puberty, like idiopathic precocious puberty, is due to a hypothalamic disorder or to a pressure effect of the thickened base of the skull on the hypothalamus. Elevated LH levels corresponding to those found during puberal development have been demonstrated in isolated cases. It is not known why this syndrome occurs almost entirely in girls and extremely rarely in boys.

The *prognosis* is good. Bone changes do not progress very much during childhood and come to a standstill during early adulthood. Spontaneous fractures usually heal well except for fractures of the upper region of the femur. As in all forms of true precocious puberty, reproductive function is normal and body height is above average during childhood and below average in adulthood.

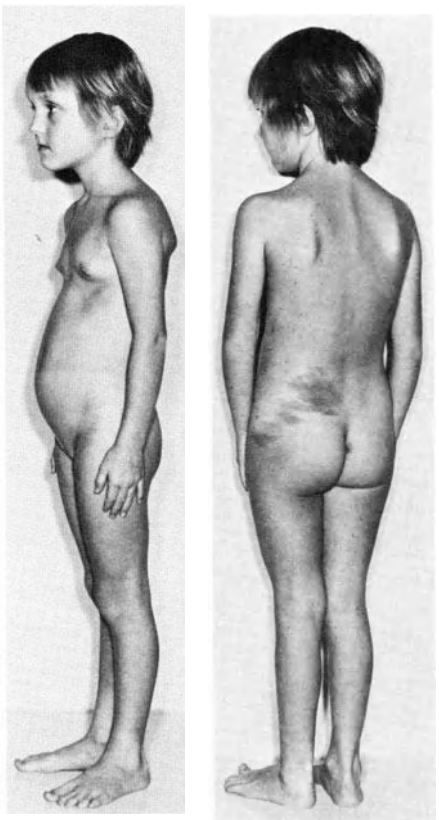


Fig. 32. Girl aged 5.5 years with the *McCune-Albright syndrome*; precocious puberty, osteodystrophia fibrosa disseminata and typical skin pigmentation (KspZ)

The *differential diagnosis* includes hyperparathyroidism (osteodystrophia fibrosa generalisata v. Recklinghausen (p. 911), xanthomatosis (Hand-Schüller-Christian), and von Recklinghausen's neurofibromatosis. A rather similar triad of symptoms also arises sometimes in neurofibromatosis, where the precocious puberty is caused by an intracranial neurinoma. Bone lesions are usually less severe, pigmentation patches are well demarcated and the appearance of cutaneous fibromas and neurofibromas confirms the differentiation.

5. Precocious Puberty due to "Hormonal Overlapping"

During the past few years, about 20 children with primary hypothyroidism (predominantly girls), some with Down's syndrome (mongoloid idiocy), and one boy with primary adrenal insufficiency and sexual precocity in addition have been reported. Puberal signs regressed in all cases with hormonal substitution therapy of the primary disorder. It is assumed that the TSH production always present secondary to primary hypothyroidism (similar to ACTH overproduction in primary adrenal insufficiency) does not remain absolutely isolated but also involves gonadotropin overproduction with corresponding sexual precocity. This phenomenon is termed "hormonal overlapping" of the hypothalamopituitary feedback mechanism (VAN WYK, 1960). It is, however, strange and puzzling that overlapping of this kind is so unusual and does not always occur with hypothyroidism and adrenal insufficiency.

The clinical picture is a combination of the classic symptoms of hypothyroidism and precocious puberty. Growth and bone maturation are delayed and thus contrast with the acceleration of body development usually seen with sexual precocity. The sella turcica is frequently enlarged, as in primary hypothyroidism. Estrogenic features, breast development, and menstruation are very pronounced. Androgenic features, and pubic and axillary hair are often absent. Galactorrhea has been observed in several cases (increased prolactin production). Urinary gonadotropins were only demonstrated in isolated cases. Excretion of 17-ketosteroids was hardly increased.

6. Precocious Puberty with Gonadotropin-Producing Tumors

Gonadotropin-producing tumors are uncommon and practically always highly malignant. Chorionepitheliomas, teratomas and malignant hepatomas secrete gonadotropins. Gonadotropin excretion, particularly LH excretion, is usually elevated and varies between normal adult values and values found in pregnancy (pregnancy test positive). The excretion of 17-ketosteroids is low or only slightly elevated.

Teratomas and chorionepitheliomas occur in both sexes and are found in the ovary or extragenitally, e.g. in the mediastinum or even in the brain.

Malignant hepatomas with sexual precocity have so far been observed only in boys. The enlarged and firm, smooth, or nodular liver leads to the diagnosis. The testes are not enlarged or only slightly, and contain Leydig cells. Tubular maturation may be absent or incomplete. Removal of the tumor containing gonadotropin results in the regression of gonadotropin excretion and of secondary sexual characteristics, but death usually follows within a few months due to lung metastases.

7. Pseudosexual Precocity with Disorders of the Adrenal Cortex

This condition is usually due to the adrenogenital syndrome which leads to isosexual pseudosexual precocity, in girls and to heterosexual pseudoprecocity in boys. It has been discussed fully elsewhere (p. 358). Pseudosexual precocity due to adrenogenital tumors is extremely rare in boys and girls (p. 375).

8. Pseudosexual Precocity with Ovarian Tumors and Cysts

Granulosa cell tumors (p. 634) are the most common ovarian tumors inducing pseudosexual precocity. They are usually unilateral and not very malignant at this age, and vary between the size of a pea and that of a child's head. The histology shows all transitional forms of luteinized (pure?) granulosa and theca cell tumors. When luteinization is very marked, the term luteoma is used.

These girls appear to be clinically healthy. All the secondary sexual characteristics arise in quick succession. Although these tumors theoretically produce predominantly estrogen (and some progesterone), pubic hair also appears prematurely. This feature may be due to the small amounts of androgens secreted by the tumor itself or to adrenarche (p. 1038) induced by the estrogens. Menstruation is often irregular but is sometimes so regular that a true menstrual cycle can be simulated. Apart from rare cases where the tumor can be palpated in the abdomen, the clinical diagnosis is based on rectal examination, but this is not very reliable if tumors are very small. The diagnosis may occasionally be suspected from the development of ascites before the tumor can be palpated rectally.

Estrogen excretion in the urine is sometimes extremely high (as in pregnancy) but is sometimes in the normal puberal range. 17-ketosteroids are usually slightly elevated. Demonstration of pregnanediol indicates a luteinized tumor.

Laparotomy is indicated where there is the slightest suspicion of this type of tumor. Bleeding occurs a few days after removal of the tumor as a reaction to the sudden withdrawal of estrogens. Menstruation subsequently

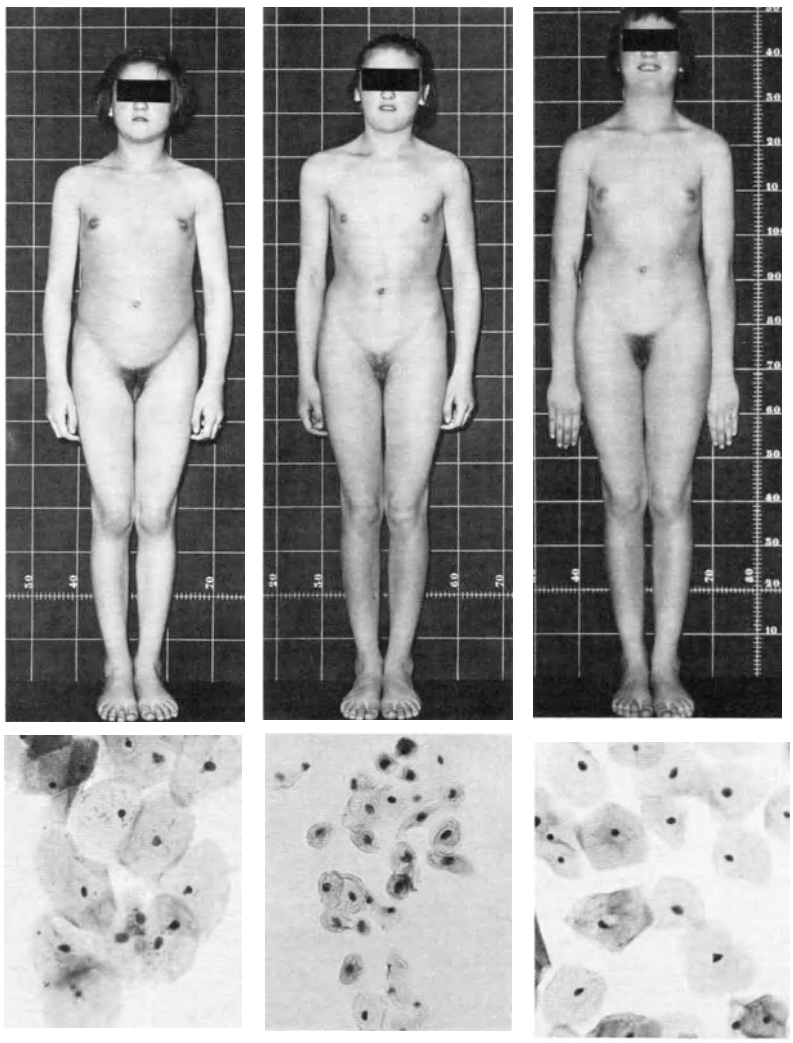


Fig. 33. Pseudosexual precocity due to a granulosa cell tumor. (The lower part of the Fig. shows the vaginal smear.) Left, at the age of 9, immediately before the operation. All the signs of puberty are recognizable. Center, one year after, partial regression of mammillae and pubic hair, and findings in vaginal smear. Another year later later (right) at the age of 11, development of normal puberty (KspZ)

ceases. Secondary sexual characteristics regress slowly, but usually not entirely. The prognosis is good in most cases.

Other ovarian tumors are occasionally also found to cause an isosexual precocious puberty. Teratomas, chorionepitheliomas (p. 688) and dysgerminomas are in this group. Androgen-secreting tumors of the ovary associated with heterosexual precocity (p. 638) have also been described several times.

Some authors also consider *follicular cysts* a cause of precocious puberty, but this association has not as yet been confirmed. Removal of the affected ovary results in regression of secondary sexual characteristics only in some cases. The other cases seem to be more similar to true idiopathic sexual precocity. Exactly the same type of follicular cyst is also found in normal ovaries.

9. Pseudosexual Precocity with Testicular Tumors

Pseudosexual precocity due to a *Leydig cell adenoma* is not very uncommon. The unilateral irregular testicular enlargement is characteristic. Excretion of 17-ketosteroids is sometimes only slightly elevated and sometimes excessively high (3–520 mg/24 h). This suggests that the metabolism of these tumors is not uniform. In the presence of high values, the adrenogenital syndrome due to adrenal hyperplasia with hyperplastic ectopic adrenocortical nodules in the testicular region must be suspected (p. 366). Excretion of 17-ketosteroids can be elevated in both conditions, unilateral irregular testicular enlargement can be present in both, and histological differentiation of Leydig cells from

adrenocortical cells is difficult. If cortisone therapy produces a reduction in the size of the testis containing the tumor and a regression of urinary 17-ketosteroids and if pregnanetriol excretion is high, the condition is more likely to be an adrenogenital syndrome.

Surprisingly, removal of the tumor does not result in regression of the clinical symptoms and reduction of 17-ketosteroids to prepuberal values in all cases. There are two possible explanations for non-correction of the symptoms. Either bone age is so far advanced that true puberty with normal testicular maturation and corresponding testicular enlargement has occurred or an adrenogenital syndrome has been missed.

10. Pseudosexual Precocity of Exogenous Etiology

It is obvious from our knowledge of the actions of gonadotropic hormones and sexual hormones that secondary sexual characteristics can arise prematurely during treatment with these hormones during childhood. Male-type puberal characteristics arise particularly when too high doses of anabolic steroids (for dwarfism) and chorionic gonadotropin (for cryptorchidism) are used. Female features are seen often with the use of skin creams containing estrogens or hair spirits with estrogens, and also when a mother uses a cream containing estrogens for her breasts and it is unintentionally acquired by the baby during breast-feeding. In rare cases secondary sexual characteristics also result from handling stilbene compounds used for castrating birds and from the intake of vitamins and drugs which have been accidentally contaminated with estrogens during manufacture. Not only the appearance of female secondary sexual characteristics is observed with the accidental intake of estrogens, but pigmentation of the mammillae and linea alba is accentuated and pubic hair arises. Any explanation of the last feature must assume that adrenarche is induced by estrogens.

11. Differential Diagnosis

Precocious puberty can usually be recognized without any difficulty. In *premature pubarche* (p. 1040) and *premature thelarche* (p. 1041), in contrast to precocious puberty, only one secondary sexual characteristic is found and hormonal findings in the urine are normal. *Vaginal bleeding in infancy* in the absence of other symptoms is only rarely endocrine in nature. Much more commonly the bleeding is due to a foreign body in the vagina, local damage, small benign tumors, or a hemorrhagic diathesis, or there has been a mistake and the bleeding is actually from the urethra or rectum. Thorough inspection of the vagina under a general anesthetic and a vaginal smear to see whether any estrogenic effects are present are often sufficient for a diagnosis.

Greater difficulties are encountered in the differential diagnosis of the etiology of precocious puberty.

The *case history* alone occasionally gives some indication of the etiology. Where there is a familial incidence, true idiopathic precocious puberty and the congenital adrenogenital syndrome must be considered. Heterosexual development of the sisters of a male patient can only occur in the adrenogenital syndrome. A family history of neurofibromatosis suggests that an intracranial neurinoma is the cause of the precocious puberty. Leading questions about cerebral symptoms (headache, vomiting, visual disorders) must be asked when the personal history is taken because of the possibility of a brain tumor. The doctor should also make a point of asking about any therapeutic or accidental contact with exogenous hormones. Slowly developing puberty is suggestive of idiopathic precocious puberty, whereas a very rapid development always suggests a tumor as the cause.

The *general examination* immediately singles out heterosexual precocity, which is always associated with an adrenocortical disorder (Fig. 34). Most cases of precocious puberty due to cerebral disorders are recognized from the neurologic examination (p. 1048). Observation of the skin pigmentation is also of importance. Smooth, well-defined pigmentation areas with subcutaneous nodules suggest sexual precocity due to neurofibromatosis, whereas patches of pigmentation with irregular borders associated with facial asymmetries or bone deformities suggest precocious puberty with fibrous bone dysplasias (Albright's syndrome).

No real conclusions about the etiology of precocious puberty can be made from the age at which the first symptoms appear and the degree of the advance in somatic development. In investigating the etiology it is essential to assess *gonadal function* as precisely as possible. This also applies to cases in which the cause of the precocious puberty seems apparent from the case history and the general examination alone. Gonadal function is assessed by the 17-ketosteroid excretion, the levels of gonadotropins, and whenever possible, also testosterone or estrogen levels in serum and urine. A positive Ascheim-Zondek test suggests a tumor-producing chorionic gonadotropin, and

in girls with true sexual precocity, the possibility of a pregnancy must be considered.

In boys, testicular size (Table 10) is easy to assess (Chap. XVIII). A unilateral, irregular enlargement is of particular significance since it suggests the presence of a tumor. Excretion of 17-ketosteroids is only slightly elevated in true sexual precocity, whereas it is very high in adrenocortical and gonadal pseudosexual precocity. If differential diagnosis is not possible with the methods so far described the action of cortisone on the excretion of 17-ketosteroids (Chap. XVIII) and the testicular biopsy (Table 10) may be helpful. The possibility of incipient brain tumor causing few symptoms must never be forgotten.

In girls (Table 9) careful rectal examination under a general anesthetic is necessary and when possible should be combined with laparoscopy for assessment of the size of the uterus and exclusion of the possibility of an ovarian tumor. The vaginal smear for assessment of the estrogenic action and estimation of the 17-ketosteroids must never be omitted. These investigations very often give no definite indication of the classification of the sexual precocity, in which case true idiopathic precocious puberty must be considered present by a process of elimination. It must always be borne in mind, however, that cases of clinically silent brain tumors and of nonpalpable ovarian tumors can be concealed under this diagnosis. A decision can often only be reached after regular follow-up examinations.

12. Treatment

From a superficial point of view, any treatment appears superfluous in *hypothalamic precocious puberty* of the idiopathic type or associated with fibrous bone dysplasia, since the advanced development produces no unfavorable effects except premature arrest of growth. The psychological problems are usually so pressing, however, that an effective form of therapy would be much appreciated. The ideal preparation would be capable of arresting premature activation of the hypothalamic-pituitary-gonadal axis, thus preventing all features of precocious puberty, without exerting any peripheral androgenic or estrogenic actions at the same time.

Trials with gestagenic drugs inhibiting gonadotropins have so far been only partly successful and it is debatable whether long-term use of these compounds in the high doses necessary is harmless and justifiable. The drugs most commonly in use are medroxyprogesterone acetate (p. 553) (Depot Provera, 100 to 200 mg/week, i.m.) and chlormadinone acetate (Gestafortin, 5 to 15 mg/day p.o.). These doses suppress secondary estrogenic sexual characteristics (menstruation, mamma development) quite efficiently in idiopathic precocious puberty and even in cases of hamartomas. Androgenic features are only partially suppressed (sexual hair, acne, masturbation). In most cases, however, these drugs do not unequivocally arrest acceleration of growth and bone maturation. There have been several reports that medroxyprogesterone acetate reduces previously elevated values of LH, FSH and testosterone in the plasma. Unfortunately it also has a mild cortisone action and thus suppresses ACTH.

Trials with the new anti-androgenic drug, cyproterone acetate (50–100 mg/day p.o.) have yielded similar results (p. 472), but it is still too early to draw any conclusions about this therapy. Cyproterone acetate reduces the con-

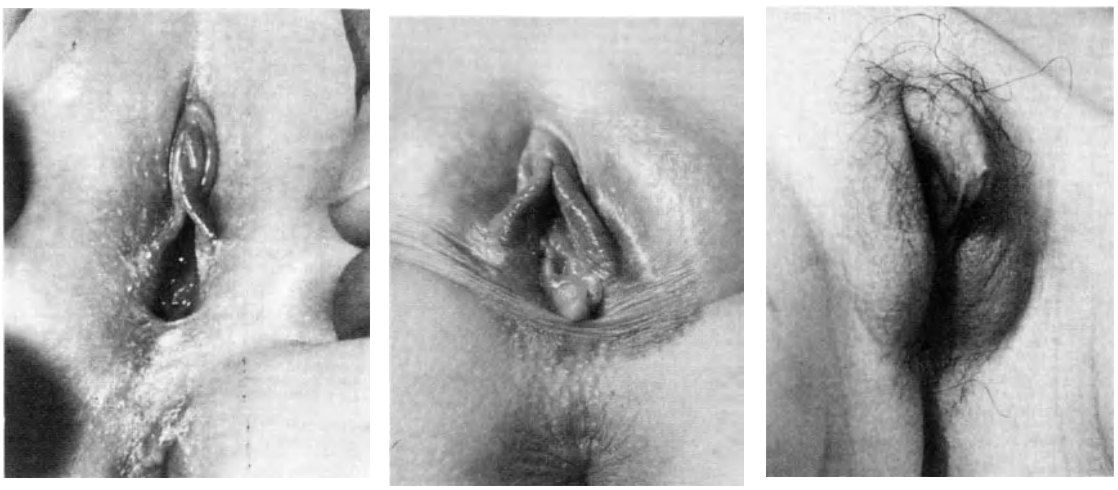


Fig. 34. Genitalia in girls during infancy. Left, normal. Center, labia minora enlarged due to true isosexual precocious puberty. Right, clitoris enlarged due to heterosexual pseudosexual precocity with an adrenogenital syndrome (KspZ)

centrations of gonadotropin and testosterone in the plasma and urine, whereas cyproterone has only peripheral anti-androgenic actions and is thus less suitable for treating precocious puberty.

In *cerebral precocious puberty*, neurosurgical intervention (excision of the tumor) is occasionally successful. Usually, however, the cerebral lesions are inoperable tumors or malformations, and only a palliative neurosurgical operation followed by radiation is possible.

For *gonadotropin-producing tumors*, the only possible treatment is immediate surgical removal of the tumor followed by radiation. The prognosis remains poor, however, due to the malignancy of the tumors.

Treatment of adrenocortical pseudosexual puberty is very rewarding, whether it involves removal of an adrenal tumor or treatment of adrenal hyperplasia with cortisone (p. 373).

Removal of the tumor (hemicastration) is the treatment of choice for *gonadal precocious pseudopuberty*. The prognosis is usually quite good since these tumors are often benign.

Precocious puberty due to *hormone overlapping* usually disappears spontaneously if the underlying endocrine disorder is correctly treated.

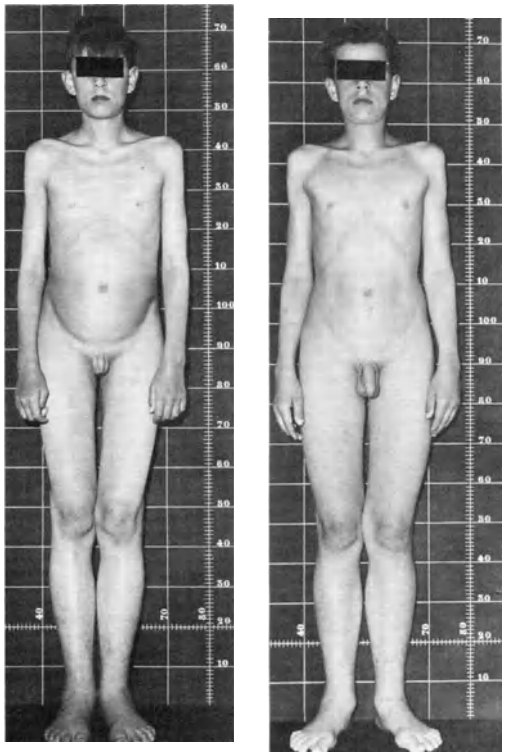


Fig. 35. Delayed puberty without generalized developmental retardation in a boy with hyperthyroidism. Left, at the age of 16.5 years, normal height but puberty absent and proportions definitely eunuchoid. The bone age of 12.5 years indicates that puberty is imminent. Right, at the age of 17 years 10 months. Spontaneous development of puberty after treatment with methimazole (Tapazole) (PRADER, 1955)

H. Delayed Puberty

The term "delayed puberty" is applied when the first secondary sexual characteristics appear after the age of 14 in girls and after the age of 16 in boys.

If no signs of puberty are present at this point, the cause of the delay must be thoroughly investigated. No endocrinopathy can be demonstrated in most cases, however, so that a chronologically displaced but otherwise normal puberty can be expected. Detailed investigation of the state of development, particularly of the height and bone age and of the often eunuchoid proportions (Fig. 35) is of the utmost diagnostic and prognostic importance. Bone age is significant since puberty normally arises at a bone age between 10.5–11.5 years in girls and between 12.5–13.5 in boys (p. 1023). If this stage of bone development has not yet been reached, puberty cannot yet be expected, but in delayed puberty where no endocrinopathy has been found, the point at which puberty can be expected can be predicted quite accurately from the bone age.

In pituitary dwarfism with gonadotropin deficiency, puberal characteristics never occur and bone age just reaches the puberal phase. In pituitary dwarfism without gonadotropin deficiency, puberty is delayed in keeping with the retarded bone maturation but is otherwise normal. In other endocrinopathies (Turner's syndrome, gonadal dysgenesis (p. 721), idiopathic eunuchoidism (p. 469), and anorchia (p. 461)) characterized by the absence of puberty, sparse pubic hair arises but the other secondary sexual characteristics fail to develop. The pubic hair is caused by adrenocortical androgens (see adrenarche, p. 1038). Bone development is retarded but gradually reaches the normal or almost normal adult state. The difference is explained by the fact that in panhypopituitarism, not only gonadotropins are absent but also growth hormone, TSH and ACTH, all of which contribute directly or indirectly to skeletal maturation (p. 1017).

The various forms of delayed puberty have been discussed in the sections of dwarfism (p. 1027), Turner's syndrome (p. 713), and pure gonadal dysgenesis, as well as in the chapters dealing with the pituitary, testis and ovary. This section gives only a survey indicating the differential diagnosis (Table 13). It must be remembered, however, that true endocrino-

pathies in which puberty fails to develop (sexual infantilism, 2a, f, 3b, c and d in Table 10) are much more uncommon than benign variations of development where puberty is merely delayed but otherwise normal (1a, 2a, b, c, 3a, e in Table 13).

Table 13. The most important aspects of the differential diagnosis of late-onset puberty

1. *Obesity*
   a) *Prepuberal obesity in boys* in whom growth is normal or accelerated (p. 1043)
2. *Dwarfism*
   a) Hypocaloric dwarfism (p. 1028)
   b) Constitutional delay of development (p. 1028)
   c) Delay of development due to chronic disease
   d) *Hypothalamo-pituitary dwarfism without gonadotropin deficiency* (p. 98)
   e) *Hypothalamo-pituitary dwarfism with gonadotropin deficiency* (p. 101)
   f) *Turner's syndrome* in patients with purely female external appearance (p. 713)
3. *Normal growth or gigantism*
   a) *Delayed puberty* (idiopathic, p. 1054)
   b) *Idiopathic eunuchoidism* and other forms of isolated gonadotropin deficiency (p. 456)
   c) *Anorchia* and other forms of Leydig-cell insufficiency (p. 461)
   d) Pure gonadal dysgenesis (p. 721)
   e) Prepuberal obesity in boys (see 1a)

In *dwarfism with delayed puberty* it is difficult to differentiate between generalized developmental delay with a poor prognosis and associated with organic hypothalamo-pituitary damage (2e) or Turner's syndrome (2f) or growth hormone deficiency without gonadotropin deficiency (2d) (where the prognosis for puberty is good) and generalized developmental retardation with no primary pituitary or gonadal disorders (2a, b, c). In the presence of *normal growth*, idiopathic delayed puberty with a good prognosis must be differentiated from idiopathic eunuchoidism (3b), which has a poor prognosis.

The reader is referred to the section on dwarfism (p. 1027) for the *differential diagnosis of generalized developmental retardation*. When growth, bone development and puberty are all retarded to the same extent, a benign constitutional delay of growth and development is the most probable cause. Pituitary dwarfism and generalized developmental retardation due to malnutrition or chronic illness are much rarer.

In cases where puberty does not arise at the normal time in spite of otherwise normal growth *idiopathic delayed puberty* is usually present. This condition is basically similar to benign constitutional delay of growth and development except that hereditary gigantism is also present. The normal body height associated with retarded bone age indicates the future gigantism. More rarely, delayed puberty is due to true idiopathic eunuchoidism (p. 456) where puberty is not really delayed but permanent hypogonadotropic hypogonadism is present due to isolated failure of the gonadotropin secretion. The two forms cannot be definitely differentiated from each other by means of conventional diagnostic methods while bone age is below 13.5 years, but it may become possible in the future by means of radioimmunological estimations of LH and FSH in serum and urine. To be on the safe side, however, any form of hormonal therapy should be postponed. If this is impossible for psychological reasons, temporary substitution treatment with chorionic gonadotropin (p. 476) or testosterone (p. 475) must be instituted and continued until a bone age of about 14 years is reached. If puberty continues spontaneously after discontination of treatment, it was merely delayed, but if signs of puberty regress after discontinuation of the drugs, a permanent gonadotropin deficiency is present. At this stage, the clinical diagnosis can also be confirmed by measuring urinary gonadotropins by conventional methods.

References

Growth

General Works

CHEEK, D. B.: Human growth. Body composition, cell growth, energy, and intelligence. Philadelphia: Lea & Febiger 1968.

FALKNER, F.: Human development. Philadelphia-London: W. B. Saunders Company, 1966.

GARDNER, L. I.: Endocrine and genetic diseases of childhood. Philadelphia-London: W. B. Saunders Company 1969.

HUBBLE, D.: Paediatric endocrinology. Oxford-Edinburgh: Blackwell Scientific Publications 1969.

TANNER, J. M.: Wachstum und Reifung des Menschen. Stuttgart: Georg Thieme 1962.

VAN DER WERFF TEN BOSCH, J. J.: Somatic growth of the child. Proceedings of a Boerhaave Course for Postgraduate Medical Teaching. Leiden: H. E. Stenfert Kroese N. V. 1966.

WILKINS, L.: The diagnosis and treatment of endocrine disorders in childhood and adolescence. Springfield: Ch. C. Thomas Publisher 1965.

Normal Values

BAYLEY, N.: Growth curves of height and weight by age for boys and girls scaled according to physical maturity. J. Pediat. **48**, 187 (1956).

— PINNEAU, S.R.: Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. J. Pediat. **40**, 423 (1952).
GREULICH, W.W., PYLE, S.I.: Radiographic atlas of skeletal development of the hand and wrist. Standford: Standford University Press 1950.
HEIMENDINGER, J.: Die Ergebnisse von Körpermessungen an 5000 Basler Kindern von 2–18 Jahren. Helv. paediat. Acta **19**, Suppl. 13 (1964).
PRADER, A.: Pathologie des Wachstums und der endokrinen Drüsen. In: FANCONI, G., WALLGREN, A., Lehrbuch der Pädiatrie, 9th ed. Basel: Schwabe & Co. 1971.
— PERABO, F.: Körperwachstum, Knochen- und Zahnentwicklung bei den endokrinen Erkrankungen im Kindesalter. Helv. paediat. Acta **7**, 517 (1952).
SCHONFELD, W.A.: Primary and secondary sexual characteristics: a study of their development in males from birth through maturity with biometric study of penis and testes. Amer. J. Dis. Child. **65**, 535 (1943).
SIMMONS, K.: Physical growth and development. Monogr. Soc. Res. Child. Develop. **9**, No 1 (1944).
— GREULICH, W.W.: Menarcheal age and the height, weight and skeletal age of girls age 7 to 17 years. J. Pediat. **22**, 518 (1943).
TANNER, J.M., WHITEHOUSE, R.H., TAKAISHI, M.: Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. I. and II. Arch. Dis. Childh. **41**, 454, 613 (1966).
VAN WIERINGEN, J.C.: Secular changes of growth. 1964–1966 Height and weight surveys in the Netherlands, in historical perspective. Netherlands Institute for Preventive Medicine TNO, Leiden 1972.

Growth and Hormones

British Medical Journal: Corticoid therapy and growth. **1**, 393 (1969).
CHEEK, D.B., GRAYSTONE, J.E., READ, M.S.: Cellular growth, nutrition and development. Pediatrics **45**, 315 (1970).
— HILL, D.E.: Muscle and liver cell growth: role of hormones and nutritional factors. Fed. Proc. **29**, 1503 (1970).
CROOKE, A.C.: Endocrine glands and growth. Schweiz. med. Wschr. **83**, 194 (1953).
EHRENGUT, W.: Morbus Addison und Wachstum. Helv. paediat. Acta **11**, 63 (1956).
PRADER, A.: Der Einfluß der Nebennierenrinde auf Wachstum und Geschlechtsentwicklung. Mschr. Kinderheilk. **104**, 157 (1956).
RAY, R.D., ASLING, C.W., WALKER, D.G., SIMPSON, M.E., LI, C.H., EVANS, H.M.: Growth and differentiation of the skeleton in thyroidectomized-hypophysectomized rats treated with thyroxin, growth hormone, and the combination. J. Bone Jt Surg. A **36**, 94 (1954).
SALTER, J., BEST, C.H.: Insulin as a growth hormone. Brit. med. J. **1953II**, 353.
THORN, G.W.: The effects of glucocorticoids upon growth and development. Schweiz. med. Wschr. **100**, 1625 (1970).
ZACHMANN, M.: Long term corticosteroid treatment and growth. Respiration **27**, Suppl., 244 (1970).
— PRADER, A.: Interactions of growth hormone with other hormones. In: STUART-MASON, A. (Ed.), Human growth hormone. Heineman Medical Books (in print) 1971.

Retardation of Growth

BIERICH, J.R., BRODT, B., GUPTA, D., SCHÖNBERG, D.: Über die konstitutionelle Entwicklungsverzögerung. Mschr. Kinderheilk. **120**, 334 (1972).
GARDNER, L.I.: Deprivation dwarfism. Sci. Amer. **227**, 76 (1972).
GHOSH, S., VARMA, K.P.S.: Progeria: Report of a case with review of the literature. Indian Pediatrics **1**, 146 (1964).
PATTON, R.G., GARDNER, L.I.: Influence of family environment on growth: The syndrome of "maternal deprivation". Pediatrics **30**, 957 (1962).
PIMSTONE, B.L., WITTMANN, W., HANSEN, J.D.L., MURRAY, P.: Growth hormone and Kwashiorkor. Role of protein in growth-hormone homoeostasis. Lancet **1966II**, 779.
POWELL, G.F., BRASEL, J.A., BLIZZARD, R.M.: Emotional deprivation and growth retardation simulating idiopathic hypopituitarism. I. Clinical evaluation of the syndrome. New Engl. J. Med. **276**, 1271 (1967).
— — RAITI, S., BLIZZARD, R.M.: Emotional deprivation and growth retardation simulating idiopathic hypopituitarism. II. Endocrinologic evaluation of the syndrome. New Engl. J. Med. **276**, 1279 (1967).
PRADER, A.: Die benigne Verzögerung von Wachstum und Pubertät und deren Abgrenzung von echten Endokrinopathien. 76. Kongreß d. Deutschen Gesellschaft für innere Medizin. Wiesbaden, April 1970, Ref. 75.
— Pathologie des Wachstums und der endokrinen Drüsen. In: FANCONI, G., WALLGREN, A., Lehrbuch der Pädiatrie, 9th ed. Basel: Schwabe & Co. 1971.
RAPPAPORT, R.: Le diagnostic des retards pubertaires. Ann. Pédiat. **44**, 313 (1968).
ROSSI, E.: Über einen Fall von Progeria. Helv. paediat. Acta **6**, 165 (1951).
SMITH, D.W.: Compendium on shortness of stature. J. Pediat. **70**, 463 (1967).
— Recognizable patterns of human malformation. Genetic, embryologic and clinical aspects. Philadelphia-London-Toronto: 1970.
THOMSON, J., FORFAR, J.O.: Progeria (Hutchinson-Gilford-syndrome). Report of a case and review of the literature Arch. Dis. Childh. **25**, 224 (1950).
VILLEE, D.B., NICHOLS JR., G., TALBOT, N.B.: Metabolic studies in two boys with classical progeria. Pediatrics **43**, 207 (1969).

Gigantism

BEJAR, R.L., SMITH, G.F., PARK, S., SPELLACY, W.N., WOLFSON, S.L., NYHAN, W.L.: Cerebral gigantism: Concentrations of amino acids in plasma and muscle. J. Pediat. **76**, 105 (1970).
MILUNSKY, A., COWIE, V.A., DONOGHUE, E.C.: Cerebral gigantism in childhood. A report of two cases and a review of the literature. Pediatrics **40**, 395 (1967).
SCHOEN, E.J., SOLOMON, I.L., WARNER, O., WINGERD, J.: Estrogen treatment of tall girls. Amer. J. Dis. Child. **125**, 71 (1973).
SOTOS, J.F., DODGE, P.R., MUIRHEAD, D., CRAWFORD, J.D., TALBOT, N.B.: Cerebral gigantism in childhood. A syndrome of excessively rapid growth with acromegalic features and a nonprogressive neurologic disorder. New Engl. J. Med. **271**, 109 (1964).
STEPHENSON, J.N., MELLINGER, R.C., MANSON, G.: Cerebral gigantism. Pediatrics **41**, 130 (1968).
WETTENHALL, H.N.B., ROCHE, A.F.: Tall girls: assessment and management. Aust. Paediatr. J. **1**, 210 (1965).

Normal Puberty

ANDERSEN, E.: Skeletal maturation of Danish school children in relation to height, sexual development, and social conditions. Acta paediat. scand., Suppl. **185** (1968).
MARSHALL, W.A., TANNER, J.M.: Variations in pattern of pubertal changes in girls. Arch. Dis. Childh. **44**, 291 (1969).
— — Variations in the pattern of pubertal changes in boys. Arch. Dis. Childh. **45**, 13 (1970).
TANNER, J.M.: Wachstum und Reifung des Menschen. Stuttgart: Thieme 1962.
— Growth and endocrinology of the adolescent. In: Endocrine and genetic disease of childhood, L.I. Gardner, Ed. Philadelphia-London: Saunders Co. 1969.

VAN WIERINGEN, J. C., WAFELBAKKER, F., VERBRUGGE, H. P., DE HAAS, J. H.: Groeidiagrammen Nederland 1965. Groningen: Wolters-Noordhoff n. v. 1968.

Hormones in Puberty

AUGUST, G. P., GRUMBACH, M. M., KAPLAN, S. L.: Hormonal changes in puberty. 3. Correlation of plasma testosterone, LH, FSH, testicular size, and bone age with male pubertal development. J. clin. Endocr. **34**, 319 (1972).

— TKACHUK, M., GRUMBACH, M. M.: Plasma testosterone-binding affinity and testosterone in umbilical cord plasma, late pregnancy, prepubertal children, and adults. J. clin. Endocr. **29**, 891 (1969).

BAGHDASSARIAN, A., GUYDA, H., JOHANSON, A., MIGEON, C. J., BLIZZARD, R. M.: Urinary excretion of radioimmunoassayable luteinizing hormone (LH) in normal male children and adults, according to age and stage of sexual development. J. clin. Endocr. **31**, 428 (1970).

BIDLINGMAIER, F., WAGNER-BARNACK, M., BUTENANDT, O., KNORR, D.: Plasma estrogens in childhood and puberty and physiologic and pathologic conditions. Pediat. Res. 7, 901 (1973).

BLUNCK, W.: Die α-ketolischen Cortisol- und Corticosteron-metaboliten sowie die 11-Oxy- und 11-Desoxy-17-keto-steroide im Urin von Kindern. Acta endocr. (Kbh.) **59**, Suppl. 134 (1968).

BUCKLER, J. M. H., CLAYTON, B. E.: Output of luteinizing hormone in the urine of normal children and those with advanced sexual development. Arch. Dis. Childh. **45**, 478 (1970).

BURR, I. M., SIZONENKO, P. C., KAPLAN, S. L., GRUMBACH, M. M.: Hormonal changes in puberty. I. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty, testicular size, and bone age in normal boys. Pediat. Res. **4**, 25 (1970).

CATHRO, D. M., SAEZ, J. M., BERTRAND, J.: The effect of clomiphene on the plasma androgens of prepubertal and pubertal boys. J. Endocr. **50**, 387 (1971).

FOREST, M. G., MIGEON, C. J.: Percentage of testosterone, androstenedione, and dehydroisoandrosterone bound to plasma protein in preadolescent children. J. Pediat. **76**, 732 (1970).

FRASIER, S. D., HILBURN, J. M., SMITH, F. G.: Effect of adolescence on the serum growth hormone response to hypoglycemia. J. Pediat. **77**, 465 (1970).

— HORTON, R.: Androgens in the peripheral plasma of prepubertal children and adults. Steroids **8**, 777 (1966).

JENNER, M. R., KELCH, R. P., KAPLAN, S. L., GRUMBACH, M. M.: Hormonal changes in puberty: IV. Plasma estradiol, LH and FSH in prepubertal children, pubertal females, and in precocious puberty, premature thelarche, hypogonadism, and in a child with a feminizing ovarian tumor. J. clin. Endocr. **34**, 521 (1972).

KNORR, D.: Über die Ausscheidung von freiem und glucuronsäure-gebundenem Testosteron im Kindes- und Reifungsalter. Acta endocr. (Kbh.) **54**, 215 (1967).

KNORR, D. W. R., KIRSCHNER, M. A., TAYLOR, J. P.: Estimation of estrone and estradiol in low level urines using electron-capture gas-liquid chromatography. J. clin. Endocr. **31**, 409 (1970).

KULIN, H. E., GRUMBACH, M. M., KAPLAN, S. L.: Gonadal-hypothalamic interaction in prepubertal and pubertal man. Effect of clomiphene citrate on urinary follicles-stimulating hormone and luteinizing hormone and plasma testosterone. Pediat. Res. **6**, 162 (1972).

LEE, P. A., MIDGLEY JR., A. R., JAFFE, R. B.: Regulation of human gonadotropins. VI. Serum follicle stimulating and luteinizing hormone determinations in children. J. clin. Endocr. **31**, 248 (1970).

PAULSEN, E. P., SOBEL, E. H., SHAFRAN, M. S.: Urinary steroid metabolites in children. I. Individual 17-ketosteroids in children with normal sexual development. J. clin. Endocr. **26**, 329 (1966).

PENNINGTON, G. W., DEWHURST, C. J.: Hormone excretion in premenarcheal girls. Arch. Dis. Childh. **44**, 629 (1969).

RIFKIND, A. B., KULIN, H. E., RAYFORD, P. L., CARGILLE, C. M., ROSS, G. T.: 24-hour urinary luteinizing hormone (LH) and follicle stimulating hormone (FSH) excretion in normal children. J. clin. Endocr. **31**, 517 (1970).

RIVAROLA, M. A., BERGADA, C., CULLEN, M.: HCG stimulation test in prepubertal boys with cryptorchidism, in bilateral anorchia and in male pseudohermaphroditism. J. clin. Endocr. **31**, 526 (1970).

ROOT, A. W., MOSHANG JR., T., BONGIOVANNI, A. M., EBERLEIN, W. R.: Concentrations of plasma luteinizing hormone in infants, children, and adolescents with normal and abnormal gonadal function. Pediat. Res. **4**, 175 (1970).

ROSENFIELD, R. L., EBERLEIN, W. R.: Plasma 17-ketosteroid levels during adolescence. J. Pediat. **74**, 932 (1969).

SAEZ, J. M., BERTRAND, J.: Studies on testicular function in children: Plasma concentrations of testosterone, dehydroepiandrosterone and its sulfate before and after stimulation with human chorionic gonadotrophin. Steroids **12**, 749 (1968).

SIZONENKO, P. C., BURR, I. M., KAPLAN, S. L., GRUMBACH, M. M.: Hormonal changes in puberty. II. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty and bone age in normal girls. Pediat. Res. **4**, 36 (1970).

SPERLING, M. A., KENNY, F. M., DRASH, A. L.: Arginine-induced growth hormone responses in children: Effect of age and puberty. J. Pediat. **77**, 462 (1970).

THOMPSOM, R. G., RODRIGUEZ, A., KOWARSKI, A., MIGEON, C. J., BLIZZARD, R. M.: Integrated concentrations of growth hormone correlated with plasma testosterone and bone age in preadolescent and adolescent males. Endocrinology **35**, 334 (1972).

WINTER, J. S. D., FAIMAN, C.: Pituitary-gonadal relations in male children and adolescents. Pediat. Res. **6**, 126 (1972).

ZACHMANN, M.: The evaluation of testicular endocrine function before and in puberty. Effect of a single dose of human chorionic gonadotropin on urinary steroid excretion under normal and pathological conditions. Acta endocr. (Kbh.) **70**, Suppl. 164 (1972).

Hypothalamus, Gonadarche, Adrenarche and Puberty

BLUNCK, W., BIERICH, J. R.: Über Frühreife. 1. Mitteilung. Physiologische Grundlagen und Untersuchungsmethoden. Mschr. Kinderheilk. **115**, 463 (1967).

CATHRO, D. M., SAEZ, J. M., BERTRAND, J.: The effect of clomiphene on the plasma androgens of pubertal and prepubertal boys. Acta endocr. (Kbh.), Abstract 204, Suppl. 138 (1969).

DONOVAN, B. T., VAN DER WERFF TEN BOSCH, J. J.: Physiology of puberty. London: Arnold 1965.

FRISCH, R. E., REVELLE, R.: Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science **169**, 397 (1970).

HARRIS, G. W., LEVINE, S.: Sexual differentiation of the brain and its experimental control. J. Physiol. (Lond.) **181**, 379 (1965).

KULIN, H. E., GRUMBACH, M. M., KAPLAN, S. L.: Changing sensitivity of the pubertal gonadal hypothalamic feedback mechanism in man. Science **166**, 1012 (1969).

— WEINSTEIN, R., KAPLAN, S. L., GRUMBACH, M. M.: The effect of high and low dose clomiphene on urinary gonadotropins and plasma testosterone in normal and XXY adult males and prepubertal children. Abstract, 52nd Meeting of the Endocrine Society (1970).

ROSENFIELD, R. L., EBERLEIN, W. R.: Plasma 17-ketosteroid levels during adolescence. J. Pediat. **74**, 932 (1969).

SOFFER, L. J., FOGEL, M., RUDAVSKY, A. Z.: The presence of a "gonadotrophin inhibiting substance" in pineal gland extracts. Acta endocr. (Kbh.) **48**, 561 (1965).

Premature Pubarche and Premature Thelarche

ARNAL, M., DRESCH, C., PRADER, A.: Etude de 8 cas de pilosité sexuelle précoce isolée ou «premature pubarche». Helv. paediat. Acta **16**, 186 (1961).

BLUNCK, W., BIERICH, J. R., BETTENDORF, G.: Über Frühreife. III. Idiopathische Pubertas praecox, temporäre Frühreife und prämature Thelarche. Mschr. Kinderheilk. **115**, 555 (1967).

COLLETT-SOLBERG, P. R., GRUMBACH, M. M.: A simplified procedure for evaluating estrogenic effects and the sex chromatin pattern in exfoliated cells in urine: Studies in premature thelarche and gynecomastia of adolescence. J. Pediat. **66**, 883 (1965).

CONLY, P. W., SANDBERG, D. H., CLEVELAND, W. W.: Steroid metabolism in premature pubarche and virilizing adrenal hyperplasia. J. Pediat. **71**, 506 (1967).

DRESCH, C., ARNAL, M., PRADER, A.: Etude de 22 cas de dévelopment prématuré isolé des seins ou «premature thelarche». Helv. paediat. Acta **15**, 585 (1960).

HELWIG, H.: Prämature Thelarche. Mschr. Kinderheilk. **113**, 581 (1965).

KENNY, F. M., MIDGLEY, A. R., JAFFE, R. B., GARCES, L. Y., VAZQUEZ, A., TAYLOR, F. H.: Radioimmunoassayable serum LH and FSH in girls with sexual precocity, premature thelarche and adrenarche. J. clin. Endocr. **29**, 1272 (1969).

PENNY, R., GUYDA, H. J., BAGHDASSARIAN, A., JOHANSON, A. J., BLIZZARD, R. M.: Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. J. clin. Invest. **49**, 1847 (1970).

THAMDRUP, E.: Premature pubarche, a hypothalamic disorder? Report of 17 cases. Acta endocr. (Kbh.) **18**, 564 (1955).

VISSER, H. K. A., DEGENHART, H. J.: Excretion of six individual 17-ketosteroids and testosterone in four girls with precocious sexual hair (premature adrenarche). Helv. paediat. Acta **21**, 409 (1966).

ZURBRÜGG, R. P., GARDNER, L. I.: Urinary C_{19} steroids in two girls with precocious sexual hair. J. clin. Endocr. **23**, 704 (1963).

Gynecomasty of Puberty

FREILINGER, G., HOWANIETZ, L., RATH, F., WALDHÄUSL, W.: Pubertätsgynäkomastie. Dtsch. med. Wschr. **96**, 1744 (1971).

PENNY, R., GUYDA, H. J., BAGHDASSARIAN, A., JOHANSON, A. J., BLIZZARD, R. M.: Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. J. clin. Invest. **49**, 1847 (1970).

SCHMIDT-VOIGT, J.: Brustdrüsenschwellungen bei männlichen Jugendlichen des Pubertätsalters (Pubertätsmakromastie). Z. Kinderheilk. **62**, 590 (1941).

Obesity before and during Puberty

British Medical Journal, leading article: The overweight child. Vol. 2, 64 (1970).

BROOK, C. G. D., LLOYD, J. K., WOLF, O. H.: Relation between age of onset of obesity and size and number of adipose cells. Brit. med. J. **1**, 25 (1972).

BRUCH, H.: Obesity in childhood. III. Physiologic and psychologic aspects of the food intake of obese children. Amer. J. Dis. Child. **59**, 739 (1940).

— Obesity in relation to puberty. J. Pediat. **19**, 365 (1941).

CARNELUTTI, M., DEL GUERCIO, M. J., CHIUMELLO, G.: Influence of growth hormone on the pathogenesis of obesity in children. J. Pediat. **77**, 285 (1970).

FRÖHLICH, A.: Ein Fall von Tumor der Hypophysis cerebri ohne Akromegalie. Wien. klin. Rdsch. **15**, 883 (1901).

KNITTLE, J. L.: Obesity in childhood: A problem in adipose tissue cellular development. J. Pediat. **81**, 1048 (1973).

MIGEON, C. J., GREEN, O. C., ECKERT, J. P.: Study of adrenocortical function in obesity. Metabolism **12**, 718 (1963).

PARRA, A., SCHULTZ, R. B., GRAYSTONE, J. E., CHEEK, D. B.: Correlative studies in obese children and adolescents concerning body composition and plasma insulin and growth hormone levels. Pediat. Res. **5**, 605 (1972).

PRADER, A.: Hypogonadismus beim Knaben. Schweiz. med. Wschr. **85**, 737 (1955).

WARD, W. A., KELSEY, W. M.: The Pickwickian syndrome. J. Pediat. **61**, 745 (1962).

WISSLER, H., GAUTIER, R., LINDER, A.: Über die Gewichtszunahme von Kindern verschiedener Altersstufen bei Mast. Helv. paediat. Acta **9**, 524 (1954).

WOLFF, O. H.: Obesity in childhood. In: Recent advances in paediatrics, ed. by D. GAIRDNER, p. 216. London: J. & A. Churchill Ltd. 1965.

Anorexia in Puberty

BELL, E. T., HARKNESS, R. A., LORAINE, J. A., RUSSELL, G. F. M.: Hormone assay studies in patients with anorexia nervosa. Acta endocr. (Kbh.) **51**, 140 (1966).

MARSHALL, J. C., RUSSELL FRASER, T.: Amenorrhoea in anorexia nervosa: Assessment and treatment with clomiphene citrate. Brit. med. J. **4**, 590 (1971).

Pubertas Praecox

General

BIERICH, J. R.: Pubertas praecox. Päd. Fortbildungskurse **23**, 44 (1968).

FERRIER, P., SHEPARD II, T. H., SMITH, E. K.: Pediatrics **28**, 258 (1961).

JOLLY, H.: Sexual precocity. Oxford: Blackwell Scientific Publ. 1955.

MONEY, J., MEREDITH, T.: Elevated verbal IQ and idiopathic precocious sexual maturation. Pediat. Res. **1**, 59 (1967).

STÄDELI, H.: Eine psychopathologische Querschnittuntersuchung bei 9 Patienten mit idiopathischer Pubertas praecox. Helv. paediat. Acta **16**, 711 (1961).

THAMDRUP, E.: Precocious sexual development. Springfield: Ch. C. Thomas 1961.

Idiopathic Pubertas Praecox

BLUNCK, W., BIERICH, J. R., BETTENDORF, G.: Über Frühreife. III. Idiopathische Pubertas praecox, temporäre Frühreife und prämature Thelarche. Mschr. Kinderheilk. **115**, 555 (1967).

DEGENHART, H. J., VISSER, H. K. A., WILMINK, R.: Excretion and production of testosterone in normal children, in children with congenital adrenal hyperplasia, and in children with precocious puberty. Pediat. Res. **4**, 309 (1970).

ESCOMEL, E.: La plus jeune mère du monde. Presse méd. **744**, 875 (1939).

JACOBSEN, A. W., MACKLIN, M. T.: Hereditary sexual precocity: report of a family with 27 affected members. Pediatrics **9**, 682 (1952).

JUNGCK, E. D., BROWN, N. H., CARMONA, N.: Constitutional precocious puberty in the male. Report of three cases. Amer. J. Dis. Child. **91**, 138 (1956).

KENNY, F. M., MIDGLEY, A. R., JAFFE, R. B., GARCES, L. Y., VAZQUEZ, A., TAYLOR, F. H.: Radioimmunoassayable

serum LH and FSH in girls with sexual precocity, premature thelarche and adrenarche. J. clin. Endocr. **29**, 1272 (1969).
PENNY, R., GUYDA, H.J., BAGHDASSARIAN, A., JOHANSON, A.J., BLIZZARD, R.M.: Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. J. clin. Invest. **49**, 1847 (1970).

Pubertas Praecox and Hormones

BULBROOK, R.D., GREENWOOD, F.C., SNAITH, A.H.: Hormone excretion in precocious puberty in girls. Arch. Dis. Childh. **33**, 295 (1958).
DEGENHART, H.J., VISSER, H.K.A., WILMINK, R.: Excretion and production of testosterone in normal children, in children with congenital adrenal hyperplasia, and in children with precocious puberty. Pediat. Res. **4**, 309 (1970).
KENNY, F.M., MIDGLEY, A.R., JR., JAFFE, R.B., GARCES, L.Y., VAZQUEZ, A., TAYLOR, F.H.: Radioimmunoassayable serum LH and FSH in girls with sexual precocity, premature thelarche and adrenarche. J. clin. Endocr. **29**, 1272 (1969).
LEVINE, L.S., NEW, M.I., PITT, P., PETERSON, R.E.: Androgen production in boys with sexual precocity and congenital adrenal hyperplasia. Metabolism **21**, 457 (1972).
NEW, M., PITT, P., PETERSON, R.E.: Testosterone production in a 3 year old male with isosexual precocity. J. Pediat. **63**, 703 (1963).
PENNY, R., GUYDA, H.J., BAGHDASSARIAN, A., JOHANSON, A.J., BLIZZARD, R.M.: Correlation of serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) as measured by radioimmunoassay in disorders of sexual development. J. clin. Invest. **49**, 1847 (1970).

Pubertas Praecox and Organic Brain Damage

BIERICH, J.R., BLUNCK, W., SCHÖNBERG, D.: Über Frühreife. II. Frühreife bei cerebral-organischen Erkrankungen. Mschr. Kinderheilk. **115**, 509 (1967).
DAVID, M., AJURIAGUERRA, J. DE, BONIS, A.: Les pubertés précoces des tumeurs cérébrales. Sem. Hôp. Paris **33**, 3935 (1957).
KITAY, J.I.: Pineal lesions and precocious puberty: a review. J. clin. Endocr. **14**, 622 (1954).
SCHMIDT, E., HALLERWORDEN, J., SPATZ, H.: Die Entstehung der Hamartome am Hypothalamus mit und ohne Pubertas praecox. Dtsch. Z. Nervenheilk. **177**, 235 (1958).

Pubertas Praecox in Fibrous Bone Marrow Dysplasia

ALBRIGHT, F., BUTLER, A.M., HAMPTON, A.O., SMITH, P.: Syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation and endocrine dysfunction, with precocious puberty in females; report of 5 cases. New Engl. J. Med. **216**, 727 (1937).
BENEDICT, P.H.: Endocrine features in Albright's syndrome (Febrous dysplasia of bone). Metabolism **11**, 30 (1962).
— Sex precocity and polyostotic fibrous dysplasia. Amer. J. Dis. Child. **111**, 426 (1966).
HUSBAND, P., SNODGRASS, G.J.A.I.: McCune-Albright syndrome with endocrinological investigations. Report of a case. Amer. J. Dis. Child. **119**, 164 (1970).
MCCUNE, D.J., BRUCH, H.: Osteodystrophia fibrosa; report of a case in which condition was combined with precocious puberty, pathologic pigmentation of skin and hyperthyroidism, with review of literature. Amer. J. Dis. Child. **54**, 806 (1937).
MÜLLER, R.: Zur Kenntnis der fibrösen Dysplasie im Kindesalter. Mschr. Kinderheilk. **104**, 387 (1956).

Pubertas Praecox as a Result of Hormonal Overlap

BERTRAND, J., LORAS, B., SAEZ, J., FOREST, M., PERETTI, E. DE, JEUNE, M.: Puberté précoce au cours d'une suffisance surrénale chronique. Nouvel exemple d'endocrinopathie complexe par entraînement? Sem. Hôp. Paris **41**, 2892 (1965).
COSTIN, G., KERSHNAR, A.K., KOGUT, M.D., TURKINGTON, R.W.: Prolactin activity in juvenile hypothyroidism and precocious puberty. Pediatrics **50**, 881 (1972).
LARON, Z., KARP, M., DOLBERG, L.: Juvenile hypothyroidism with testicular enlargement. Acta paediat. scand. **59**, 317 (1970).
PABST, H.F., PUESCHEL, S., HILLMAN, D.A.: Etiologic interrelationship in Down's syndrome, hypothyroidism, and precocious sexual development. Pediatrics **40**, 590 (1967).
VAN WYK, J.J., GRUMBACH, M.M.: Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: An example of hormonal overlap in pituitary feedback. J. Pediat. **57**, 416 (1960).

Pubertas Praecox and Tumors Producing Gonadotropin

BRAUNSTEIN, G.D., BRIDSON, W.E., GLASS, A., HULL, E.W., MCINTIRE, K.R.: *In vivo* and *in vitro* production of human chorionic gonadotropin and α-fetoprotein by a virilizing hepatoblastoma. J. clin. Endocr. **35**, 857 (1972).
BRUTON, O.C., MARTZ, D.C., GERARD, E.S.: Precocious puberty due to secreting chorionepithelioma (teratoma) of the brain. J. Pediat. **59**, 719 (1961).
EDMONDS, L.C., CARRERA, G.M.: Extragenital choriocarcinoma. J. Pediat. **67**, 94 (1965).
HUNG, W., BLIZZARD, R.M., MIGEON, C.J., CAMACHO, A.M., NYHAN, W.L.: Precocious puberty in a boy with hepatoma and circulating gonadotropin. J. Pediat. **63**, 895 (1963).
HUTCHINSON, J.S.M., BROOKS, R.V., BARRATT, T.M., NEWMAN, C.G.H., PRUNTY, F.T.G.: Sexual precocity due to an intracranial tumour causing unusual testicular secretion of testosterone. Arch. Dis. Childh. **44**, 732 (1969).
KOSENOW, W., FEIL, W., TÖRNE, H. VON, BIERICH, J.R., APOSTOLAKIS, M.: Sexuelle Frühreife durch primäres Lebercarcinom: „Hepatogenitales Syndrom". Mschr. Kinderheilk. **115**, 37 (1967).

Pubertas Praecox and Ovarian Tumors

AMMANN, A.J., KAUFMANN, S., GILBERT, A.: Virilizing ovarian tumor in a $2^1/_2$-year-old girl. J. Pediat. **70**, 782 (1967).
EBERLEIN, W.R., BONGIOVANNI, A.M., JONES, I.T., YAKOVAC, W.C.: Ovarian tumors and cysts associated with sexual precocity. J. Pediat. **57**, 484 (1960).
REIS, R.L., KOOP, C.E.: Ovarian tumors in infants and children. J. Pediat. **60**, 96 (1962).
ZURBRÜGG, R.P., WAGNER, H.P.: Interrelations between hormonally active tumors and sexual precocity. A brief discussion basing on six illustrative case histories. Helv. paediat. Acta **25**, 99 (1970).

Pubertas Praecox and Testicular Tumors

ALLIBONE, E.C., ANDERSON, C.K., ARTHURTON, M.W.: Macrogenitosomia praecox due to an interstitial cell tumour of the testis. Arch. Dis. Childh. **44**, 84 (1969).
BISHOP, P.M., VAN MEURS, D.P., WILLCOX, D.R.C., ARNOLD, D.: Interstitial-cell tumour of the testis in a child. Report of a case and a review of the literature. Brit. med. J. **1960 I**, 238.

ROOT, A., STEINBERGER, E., SMITH, K., STEINBERGER, A., RUSS, D., SOMERS, L., ROSENFIELD, R.: Isosexual pseudoprecocity in a 6-year-old boy with a testicular interstitial cell adenoma. J. Pediat. **80**, 264 (1972).

WEICKER, H.: Der Leydig-Zelltumor. Minerva pediat. **17**, 736 (1965).

Pubertas Praecox and Exogenic Hormones

BERGER, H., BIANCHETTI, B.: Gynäkomastie und abnorme Pigmentierung durch Hexöstrol. Ther. Umsch. **17**, 119 (1960).

CURTIS, E. M.: Oral-contraceptive feminization of a normal male infant. Report of a case. Obstet. and Gynec. **23**, 295 (1964).

HERTZ, R.: Accidental ingestion of estrogens by children. Pediatrics **21**, 203 (1958).

LANDOLT, R., MÜRSET, G.: Vorzeitige Pubertätsmerkmale als Folge unbeabsichtigter Oestrogenverabreichung. Schweiz. med. Wschr. **98**, 638 (1968).

WEBER, W. W., GROSSMAN, M., THOM, J. V., SAX, J., CHAN, J. J., DUFFY, M. P.: Drug contamination with diethylstilbestrol; outbreak of precocious puberty due to contaminated isonicotinic acid hydrazide (INH). New Engl. J. Med. **268**, 411 (1963).

Treatment of Pubertas Praecox

GORDON, G. G., SOUTHREN, A. L., TOCHIMOTO, S., OLIVO, J., ALTMAN, K., RAND, J., LEMBERGER, L.: Effect of medroxyprogesterone acetate (Provera) on the metabolism and biological activity of testosterone. J. clin. Endocr. **30**, 449 (1970).

MATHEWS, J. H., ABRAMS, C. A. L., MORISHIMA, A.: Pituitary-adrenal function in ten patients receiving medroxyprogesterone acetate for true precocious puberty. J. clin. Endocr. **30**, 653 (1970).

MENKING, M., BLUNCK, W., WIEBEL, J., STAHNKE, N., WILLIG, R. P., BIERICH, J. R.: Über Frühreife. IV. Mitteilung: Therapie und Pubertas praecox. Mschr. Kinderheilk. **119**, 19 (1971).

RIFKIND, A. B., KULIN, H. E., CARGILLE, C. M., RAYFORD, P. C., ROSS, G. T.: Suppression of urinary excretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by medroxyprogesterone acetate. J. clin. Endocr. **29**, 506 (1969).

RIVAROLA, M. A., CAMACHO, A. M., MIGEON, C. J.: Effect of treatment with medroxyprogesterone acetate (Provera) on testicular function. J. clin. Endocr. **28**, 679 (1968).

TELLER, W. M., MÜRSET, G., SCHELLONG, G.: Urinary C_{19} and C_{21} steroid patterns in isosexual precocious puberty during long-term treatment with gestagens. Acta paediat. scand. **58**, 385 (1969).

Pubertas tarda

The reader is referred to the references listed under retardation of growth (p. 1029), Turner's syndrome (p. 713), pituitary dwarfism (p. 98), and hypogonadism (p. 500, 456).

XX. Fundamentals of the Hormone Treatment of Nonendocrine Disorders

A. LABHART and G. MARTZ

A. Endocrine Therapy of Carcinomas of the Breast, Prostate, and Corpus Uteri and of Prostatic Hypertrophy

1. Introduction

Whereas the proportion of patients with malignant growths within the total hospital population has increased considerably over the past 25 years, effective, albeit still only palliative, methods of treatment have now become available. Malignant tumors of endocrine-regulated organs (breast and prostate) are very common. When the surgical and radiotherapeutic possibilities have been exhausted, treatment with hormones is available for these cases, which are of interest to the endocrinologist as well as to the oncologist.

In keeping with the interest of the endocrinologist in this, perhaps the widest, use of hormones, the principles of endocrine therapy of these three neoplasias will be discussed in this chapter. The chapter is based on the monograph by one of us (MARTZ), from which the synoptic tables are also derived.

BEATSON started the era of the endocrine treatment of tumors in 1896 by performing a successful surgical castration for metastasizing breast carcinoma. His theoretical reasons have since been disproved. It was HUGGINS, in 1941, who made castration known to physicians as the most important palliative treatment for inoperable carcinoma of the prostate. He based his reasoning on critical experimental trials in animals. He recognized that two different properties of the malignant growths – the hormone dependence and the hormone sensitivity of organs under endocrine control, such as the prostate – could be exploited therapeutically. This shattered the dogma of the autonomous growth of all malignant tumors. The extremely variable results and the initially deficient theoretical bases, some of which did not at first admit of experimental documentation, have led to general uncertainty over the last 25 years. This has resulted partly in uncontrollable endocrine polypragmasia and partly in therapeutic nihilism. A reliable, although empirical, foundation for this therapy has only emerged since the advent of controlled studies on a cooperative basis. Such terms as "tumor regression," "therapeutic success," and "period of survival" have been internationally defined according to strict criteria. The basic information given here is derived from 12 years' experience of such cooperative studies, in many of which the Division of Oncology at the Kantonsspital Zurich participated.

2. General Features

Every malignant tumor of the breast, prostate or the body of the uterus must first be placed in the hands of the surgeons and radiologists, providing it is still localized. The disseminated tumor belongs in the domain of medical therapy which almost without exception still has only a palliative effect. Whereas cytostatic drugs attack the tumor directly, the endocrine measures act on the host, and thus only indirectly on the tumor. The mode of action at the cellular level is still unknown, as is the mode of most hormonal actions.

One quarter of all deaths due to malignant tumors are due to the potentially hormone-dependent carcinomas of the breast, prostate and body of the uterus. However, only a number of these malignant tumors respond to hormonal therapy and then only for a limited time. (See MARTZ, 1968, for other rare tumor types which can be influenced by hormones.)

3. Endocrine Therapy of Carcinoma of the Breast

With a morbidity of 3 %, carcinoma of the breast is the most common form of cancer leading to death. Curative treatment is impossible in about one third of patients at the time of diagnosis, and there are recurrences within 10 years in about two-thirds of those subjected to surgery. The very variable spontaneous course of carcinoma of the breast must always

be kept in mind. In addition to the types of carcinoma which can lead to death within months, there are "benign" forms with a course lasting over 20 years, so that the term "five-year survival" is obsolete. This means careful follow-up and the prospective studies mentioned above are essential for rational therapy.

Growth and function of the breast are subject to manifold hormonal influences (p. 694). Some carcinomas of the breast are hormone-dependent and others are not. The ones that are, however, always become independent after some time. There is no histological difference between the two types. Basically, *ablative* therapy is possible by the withdrawal of hormones, and *additive* therapy by the administration of hormones. Ablative therapy is generally more effective. The sequence of treatment is summarized in Table 1.

Table 1. Sequence of treatment (MARTZ, 1968)

Patient before or shortly after onset of menopause	
Initial treatment	Ovariectomy
Second measure	
a) After remission following ovariectomy	Hypophysectomy or adrenalectomy
(If surgery is not possible or is refused by the patient:	Androgens)
b) If ovariectomy has had no effect:	Chemotherapy
Third measure	Chemotherapy, corticosteroids

Patient more than 5 years after onset of menopause	
Initial treatment	Estrogens
Second measure	
a) After remission by estrogens:	Androgens or estrogens + progesterone
b) If estrogens have no effect:	Chemotherapy
Third measure	
a) If previous hormone therapy was effective:	Hypophysectomy or adrenalectomy
b) In all patients	Corticosteroids, chemotherapy

a) Treatment by Withdrawal of Hormones

The purpose of this treatment is the most extensive elimination possible of all hormones which promote the growth and spread of carcinoma of the breast.

α) Elimination of Ovarian Function

One quarter to one third of patients in the premenopause respond to ovariectomy with an objective remission lasting about 10 months on average. The effects are greatest on metastases of the soft tissues (skin, lymph nodes, breast) and of the skeleton, and perhaps also on the primary tumor itself. Metastases of the liver and central nervous system are least affected. The results are often dramatic. Metastases disappear completely, the general condition, appetite and body weight improve and, above all, bone pain disappears rapidly, often within hours after the operation. The analgesic effect is not always associated with objective remission of the metastases. There is no certain criterion allowing accurate prediction of the result of the operation. Patients shortly before or after menopause react best, whereas the chances of success are slighter in women under 30. After the menopause, the vaginal cytology and the estimation of the urinary gonadotropins can indicate how active the ovaries still are. A further criterion is the free interval between the development of the primary tumor and the appearance of metastases; the longer this free interval is, the more likely the ovariectomy is to be successful.

Indications and statistical chances of success of castration can be summarized as follows: ovarian elimination is indicated as the first endocrine measure in all patients with metastasizing carcinoma of the breast in the premenopause and up to 5 years after the menopause.

Surgical ovariectomy is preferable whenever it is possible. It has the advantage of a rapid onset of action and its success can often be assessed immediately after the operation. The elimination of ovarian function by radiation has the advantage of slighter stress to the patient, but there is a risk of mucosal lesions of the intestine. Above all, the onset of action is delayed, and the result cannot usually be assessed until 3–6 months afterwards. As a rule, a patient should only be radiated when her condition does not permit surgery. Prophylactic menolysis radiation is an exception (p. 1063). Additional endocrine treatments should definitely be avoided after ovariectomy until the succes or failure of the operation has been determined. Hot flushes due to castration should be treated with sedatives and not with hormones.

In spite of contradictory statistics, *prophylactic* ovariectomy in carcinoma of the breast, i.e. ovariectomy in the absence of demonstrable metastases, is not justified. Several rigorous prospective trials have proved the ineffectiveness of this procedure. The main argument against prophylactic castration is the fact that 30 to 50% of the patients are definitely cured by the primary surgical treatment so that this operation,

which is associated with severe psychic stress, would be needless. If axillary metastases are present prophylactic ovariectomy must be seriously considered. The decision should be made in the light of the patient's attitude and of whether she has or wishes to have children. Strictly speaking, ovariectomy is no longer a prophylactic measure in a case with metastases. However, radiation of the ovaries seems to have a beneficial effect in postmenopausal patients.

β) Elimination of the Pituitary and the Adrenal Cortex

After gonadal ablation the production of sexual hormones in the adrenals sometimes increases (p. 459). Ablation of the pituitary or adrenals should be considered as the second or third step in the endocrine therapy of metastasizing carcinoma of the breast. It leads to the complete elimination of the sexual hormones and related steroids. The best results are obtained in cases where previous castration has been effective. The analgesic effect is often immediate. Objective improvement is to be expected in 30 to 40% of cases and subjective effects in 60 to 80%.

The indications for elimination of the pituitary or adrenals are based on the following conditions:

previous response to other endocrine treatments;
painful skeletal metastases;
absence or small size of visceral metastases.

The surgical technique for adrenalectomy and the substitution therapy are discussed on p. 330, 356, and the possible methods of eliminating the pituitary by surgical, radiological, or stereotactic measures and of pituitary substitution on p. 105, 115.

After α-particle radiation, which is a complicated and expensive procedure, stereotactic isotope implantation or bipolar electrocoagulation presents the least stress for the patient. According to experience so far, the results of adrenalectomy and hypophysectomy are comparable. Prospective comparative series are in progress but cannot yet be evaluated. However, a trend in favor of hypophysectomy is beginning to appear. In contrast to stereotactic elimination of the pituitary, bilateral total adrenalectomy is a major operation.

b) Treatment by Hormone Administration

α) Androgens

About 20% of patients with metastasizing carcinoma of the breast respond to treatment with high doses of the male sexual hormones. Very high doses are necessary (100 mg testosterone propionate i.m. 3 times weekly, or 20–30 mg fluoxymesterone daily; see Table 2). They lead without exception to severe and often irreversible manifestations of virilization, which is an extremely severe psychological trauma for the patient (Table 3; p. 1064). Women of all ages respond to this therapy.

Table 2. Dosage scheme

Preparations	Dosage
1. *Estrogens*	
Diethylstilbestrol, p.o.	5 mg 3 times daily
Ethinylestradiol, p.o.	1 mg 3 times daily
Estradioldipropionate(-benzoate), i.m.	5 mg 2–3 times weekly
2. *Androgens*	
Testosterone propionate, i.m.	100 mg 3 times weekly
Testosterone ester depot i.m.	250 mg once weekly
Fluoxy mesterone, p.o.	10 mg 3 times daily
3. *Progesterone* (in combination with estrogens)	
Hydroxyprogesterone capronate, i.m.	1000 mg weekly
4. *Adrenal steroids*	
Prednisone, p.o.	10–20 mg 3 times daily
Triamcinolone, dexamethasone or similar preparations	According to the effective equivalent for prednisone (see p. 309)

The antineoplastic action is apparently coupled with the androgenic properties, and anabolic steroids only have a comparable effect when given in doses which have a virilizing effect. On the other hand, a careful study carried out on a cooperative basis with Δ-1-testololactone, an artificial steroid which has no hormonal activity, has given results as good as are obtained with testosterone. The preparation must be given in the same high doses – 100 mg i.m. 3 times weekly.

Water retention is another side effect of testosterone therapy and can be better controlled. It can be prevented by limiting the intake of salt or by giving diuretics. Other, rarer side effects are mild hypertension and erythrocythemia and exacerbation of skeletal pain in 10–20%, perhaps associated with a rise of the serum calcium. This initial stimulation of the tumor usually declines after a few days or at the most weeks of uninterrupted administra-

Table 3. Side effects of hormonal therapy (MARTZ, 1968)

Mode of action	Side effects	Therapy of side effects
Castration	Menopausal symptoms (hot flushes, etc.)	Sedatives *no hormones*
Adrenalectomy	Symptoms of endocrine insufficiency	Substitution
Hypophysectomy	Symptoms of endocrine insufficiency	Substitution
	Liquorrhea	Repeated spinal taps; surgery
	Lesion of the optic nerve	–
Estrogens	Lack of appetite, nausea	Change of preparation
	Withdrawal bleeding	Ceases spontaneously during continued medication with estrogens. If not: injection of 100 mg progesterone
	Pigmentation of the nipples	–
	Water retention	Diuretics, cardiac therapy
	Hypercalcemia	Discontinuation of therapy, low-calcium diet, intensive supply of liquids, therapy with phosphates, 100 mg prednisone/24 h
Androgens	Virilization (deep voice, growth of beard, baldness)	–
	Increase in libido	–
	Rise in blood pressure	Blood pressure-lowering preparations,
	Water retention	diuretics, cardiac therapy
Corticosteroids	Cushingoid habitus	–
	Ulcer of stomach and intestine	Antacids
	Diabetes	Diabetes therapy
	Infections	Antibiotics
	Osteoporosis	Calcium and/or fluor preparations

tion of the androgens. It is not an absolute indication for discontinuation of the therapy if the patients are in hospital under careful observation. These cases must be distinguished from those with progressively spreading metastases in hormone-insensitive carcinomas. Objective remission is noticeable after about 2 months at the earliest. Skeletal pains disappear, and skeletal and soft tissue metastases regress. The effect lasts an average of 7–10 months.

β) Estrogens

Postmenopausal women respond to estrogens more frequently and for longer periods than to androgens, i.e. in 35 % of cases as against 20 %. There is very little chance of success in younger women. Low doses are advocated at the beginning of treatment because of possible gastrointestinal intolerance. The optimal daily maintenance dose of 15 mg stilbestrol or 3 mg ethinyl estradiol should be attained within a week. All metastases can be influenced, especially those in the soft tissues. Objective remissions are determined about 2 months after the beginning of the treatment. The effects usually last longer than those obtained with androgens on average 13 months. In rare cases estrogens lead to an exacerbation of the tumor growth, which can be recognized in the presence of bone metastases from the appearance of pain.

Uterine withdrawal bleeding is the main side effect. It usually ceases spontaneously with the continuation of the treatment, or it can be arrested with high doses of progesterone. Water retention can be prevented by limitation of salt intake and administration of diuretics. Urinary incontinence and vaginal discharge occur in some cases. Hypercalcemia arises in about 10 % of cases; it is a serious complication which necessitates immediate energetic measures (p. 933, 968).

γ) Cortisol and its Derivatives (p. 303)

δ) Gestagens

The relatively slight experience so far available seems to indicate that the results with high doses of gestagens in metastasizing carcinoma of the breast are not as good as those obtained with androgens or estrogens. Some advantage may be gained by combining gestagens with estrogens. The first observations in patients receiving ovulation inhibitors indicate therapeutic and prophylactic effects for this combination. The combination of androgens and estrogens, however, does not produce any better results than are obtained with either of these hormones alone.

The combination of hormone therapy with cystostatics may prove more effective than either method alone. It cannot be assessed at present.

c) Indications and Strategy

With the possible exception of prophylactic castration in women in the menopause, endocrine therapy is only justified when metastases of the tumor can be demonstrated. Histological proof of the primary tumor is essential, and proof of easily accessible metastases is desirable. Localized metastases should be treated locally whenever possible, i.e. by surgery and/or radiation.

A trial with hormones should be avoided in patients with no symptoms and no local or generalized signs of tumor progression. Endocrine treatment should be instituted when symptoms arise or when spread of the metastases has been demonstrated. Careful supervision of each patient is an important requirement.

The most objective assessment of the metastases possible, i.e. frequent measurements of their size, is essential for successful endocrine therapy. A so-called metastatic series of 7 X-rays should always be taken (lateral skull, thorax and pelvis a.p., thoracic and lumbar vertebrae a.p. and lateral). Experience has shown that these reveal more than 90% of all skeletal metastases occurring in this condition.

In summary, the following rules should be adhered to: Therapeutic trial only after there is evidence of progression of the metastases or of symptoms due to the tumor. Measurement and recording of all direct and indirect manifestations of the tumor present before and during the treatment. Never more than *one* hormonal measure at the same time. Continuation of the measure introduced until renewed growth of the tumor can be objectively confirmed. Hormone therapy with estrogens or androgens, once started, should be continued in the same doses until a recurrence can be demonstrated under continuing treatment. At this point, the hormone must be completely withdrawn and no further treatment started. An observation period must then follow. In some cases, the abrupt withdrawal of high doses of estrogens or androgens can bring about an inhibitory effect on the tumor once more, so that a remission arises as a result of the withdrawal alone. This withdrawal effect, which is not, however, common, should be watched for before subsequent introduction of a different treatment (Table 1).

Prognosis. With the exception of the length of the free interval (p. 1062), there is at present no means of predicting the chances of success of an endocrine therapy. The histology of the tumor or the metastases gives no indication. The laboratory tests have so far failed entirely, even the calciuria test after estrogens and the sex chromatin in the tumor tissue. Refer to MARTZ (1968) for further tests (P_{32} uptake by HALE, discriminants of BULBROOK [relative amounts of the excreted steroids]). See Tables 1, 2 and 3 for the plan of treatment, dosages, side effects and countermeasures.

4. Endocrine Therapy of Metastasizing Carcinoma of the Breast in the Male

The incidence of carcinoma of the breast in men is 1% of that in women. There is no connection with gynecomastia. Orchidectomy, as the most effective therapeutic measure, is the treatment of choice; it probably achieves a higher percentage of remissions (which are also of longer duration) than is obtained in women receiving endocrine therapy. Adrenalectomy and hypophysectomy have to be considered when there are recurrences. Estrogens are only effective in isolated cases. Treatment with cortisol can be tried as a last resort in this condition.

5. Endocrine Therapy of Carcinoma of the Prostate

Carcinoma of the prostate is the most common type of malignant neoplasia in men over 50 in the Western Hemisphere except for skin carcinoma. Only an average of 10% can be treated by radical surgery at the time the diagnosis is made. Palliative treatment is therefore of great importance. Aims of the treatment are correction of symptoms associated with micturition, removal of pain, improvement of the general condition and prolongation of life. An objective assessment of the status before and during treatment is esssential for the administration of an optimal therapy in this condition also. Estimation of the acid phosphatase in the serum often gives an indication of the extent of the metastases.

It must be observed at this point, however, that for some unknown reason 50% of cases of metastasizing carcinoma of the prostate are not associated with a rise of the acid phosphatase.

a) Therapy by Withdrawal of Hormones

Orchidectomy, i.e. the withdrawal of androgen production, leads to atrophy of the healthy as well as of the neoplastically transformed prostate. It subjects the patient to little stress, even in the advanced age group. But castration can cause severe psychological trauma. Impotence and loss of libido can (but need not) arise.

When the effects of orchidectomy wear off, the tumor increases in size and the symptoms worsen. In general nothing more can be expected of treatment with estrogens after this. The possible alternatives are adrenalectomy and hypophysectomy to eliminate the increased androgen production by the adrenals which follows castration. In elderly patients stereotactic elimination of the pituitary, which involves only slight stress to the patient, is the procedure usually considered. It promises at least an analgesic action.

b) Estrogen Therapy

The optimal dose of estrogens for this condition is not known. It is probably less than that for carcinoma of the breast, and the lowest effective dose should be used. Two methods can be recommended. An intramuscular injection of a depot estrogen preparation such as polyestradiol phosphate in a dose of 40–80 mg every 2 to 4 weeks, or oral administration of 1–3 mg ethinyl estradiol or 5–15 mg diethylstilbestrol daily. The actions of diethylstilbestrol diphosphate correspond to those of the other estrogen preparations. Estrogen therapy brings about the disappearance of bone pain and an improvement in general condition in 70–80% of patients. There is an increase in body weight and hemoglobin, and the primary tumor decreases in size, so that the patient can often be relieved of the indwelling catheter. The prolongation of survival is not known. Regression of the skeletal metastases is only occasionally seen on X-rays.

Metastases in the soft tissues can disappear. Undesirable side effects are loss of libido, impotence, feminization, and hot flushes, and also the syndrome of the male climacteric. The painful gynecomastia which accompanies estrogen treatment can be prevented by previous radiation of the breasts. Retention of NaCl and water, which can be dangerous in the presence of circulatory failure, can be prevented by limiting the salt intake and giving diuretics or digitalis.

Estrogen therapy should only be employed in inoperable carcinoma of the prostate which causes symptoms. The primary tumor can regress and become operable under treatment with estrogens. Estrogen therapy is indicated when metastases are demonstrated, whenever possible histologically or by elevated acid phosphatase, and when metastases cause symptoms.

c) Treatment with Cyproteron

The synthetic anti-androgenic steroid cyproteron or cyproteron acetate (p. 472) can block the peripheral action of androgens completely when given in adequate doses (p. 472). Therapeutic trials in prostatic carcinoma have been favorable so far. A broad prospective study is in progress. Cyproteron has one advantage over the estrogens in that it has no feminizing action, but there is usually a loss of libido and potency.

d) Cortisol and Derivatives

The glucocorticoids are thought to be as effective as hypophysectomy and adrenalectomy in carcinomas of the prostate which no longer react to estrogens or orchidectomy. This procedure is therefore preferable when the advanced age of the patient must be taken into account (p. 1069).

e) Gestagens

Gestagens are also thought to act on prostatic carcinoma, but this therapy is still in the trial stages.

f) Therapeutic Strategy

Histological proof of carcinoma is an essential condition for any specific therapy. The extent of the disease should then be estimated by means of palpation, radiology and chemical analysis of the blood. Age, general condition, the most optimistic life expectancy, and the psychological factors must be carefully weighed against the symptoms caused by the prostate carcinomal and against the side effects expected to result from endocrine treatment. Younger patients are advised to undergo orchidectomy providing it can be tolerated psychologically. Estrogens, cortisol and its derivatives and possibly cyproteron should be considered for older patients. It is sometimes best to delay endocrine treatment in elderly patients with no symptoms, even if there are extensive metastases.

Local urological measures and the radiation of painful metastases must not be neglected.

Once an endocrine therapy has been chosen, it must be continued until definite signs of renewed tumor growth are recognized.

When the estrogen therapy which was successful at first fails, another remission can often be obtained with orchidectomy. As has been mentioned above, estrogen therapy has no chance of success after primary castration. A further response can also be achieved by increasing the dose of the estrogen preparation by 10 times or more or by changing the type of estrogen preparation. Cortisone derivatives and progesterone can also be considered, and a favorable effect is paradoxically produced

with androgens in isolated cases. Finally, there are cytostatics such as cytoxan or fluorouracil and P_{32}.

6. Endocrine Therapy of Carcinoma of the Body of the Uterus

The frequency of carcinoma of the body of the uterus appears to be increasing, although the tumor is less common than carcinomas of the breast and the prostate. Surgery and radiotherapy are successful in 60–70% of cases. The inoperable or metastasizing cases, however, usually have a rapid progressive course. Endocrine disorders appear to be common in patients with carcinoma of the body of the uterus, and there is an obvious tendency for cystic and adenomatous hyperplasia of the mucosa of the body to become malignant in 10% of cases due to untimely or increased estrogen stimulation.

NATHANSON in 1960 was the first to achieve objective tumor regression, in some cases lasting several years, in one third of 22 patients with high doses of progesterone. It has since been confirmed that about 30% of all cases respond to high doses of progesterone. The mode of action is unknown.

As in carcinoma of the breast, a long free interval between surgical removal of the primary tumor and the development of metastases is the only indication of a probable favorable response to endocrine therapy. This treatment seems to act most favorably on metastases of the lungs and bones. Local tumor recurrences respond less well. Hormone therapy cannot be used for undifferentiated carcinomas or for sarcomas.

The optimal dose is still not known. It is assumed that a dose of 500 mg 17-alpha-hydroxyprogesterone capronate given by i.m. injection twice weekly is adequate for lung metastases. Doses of 2–5 g per week are necessary for skeletal, abdominal, and pelvic metastases. Medroxyprogesterone acetate is a depot preparation and should be given in a dose of 1–3 g i.m. every week or of 200–300 mg p.o. daily. The initial doses must be high and should then be gradually reduced. The therapeutic effect can only be assessed after about 2 months. The treatment must be continued without interruption until it fails. An increase in the dose or a change of the drug may then bring about a further remission. In some patients progesterone therapy can cause an inoperable tumor to become operable.

In contrast to other endocrine therapies, progesterone causes practically no side effects. Mild loss of appetite and nausea occur rarely and are of short duration. Progesterone preparations should therefore be considered for the prophylaxis of metastases, but no controlled studies relating to this application have yet been published.

7. Endocrine Treatment of Prostatic Hypertrophy

Prostatic hypertrophy or the benign adenoma of the prostatic gland can also be influenced and treated palliatively by hormones, but hormonal treatment is no substitute for prostatectomy and should only be considered if surgery is contraindicated.

The subjective improvements which can be induced by androgen treatment are probably nonspecific and are based on increased tonus of the muscles of the bladder. If carcinoma of the prostatic gland can be excluded, it is permissible to give men under 55 25 mg of testosterone propionate twice weekly for 6 weeks. This medication can be repeated not more than twice a year. Estrogens inhibit secretion and the swelling of the epithelial parts of the adenoma. Micturition can be improved by 5 mg diethylstilbestrol p.o. daily, but the side effects of this treatment are loss of potency and gynecomastia.

The anti-androgen cyproteron acetate blocks the effect of testosterone (p. 472) without feminization. The usefulness of this preparation is still in the trial stage.

There have recently been reports of improvements brought about by gestagens (GELLER, 1965; VERNET, 1970).

B. Pharmacological Use of Hormones

1. Cortisol and its Derivatives

Since HENCH'S observation of a definite effect of cortisone on rheumatoid arthritis in 1949, cortisol derivatives have also been used extensively outside the field of endocrinology because of the following effects:

1. Anti-inflammatory effect;
2. Immunosuppression (anti-allergic effect);
3. Palliative antineoplastic effect;
4. Antihypercalcemic effect;
5. Various other, mostly unexplained effects.

a) Anti-Inflammatory Action

Cortisol and its derivatives in doses four to twelve times greater than required for substitution therapy usually have a definite inhibitory action on inflammation. An effect can also be achieved in certain cases with small

doses which, when divided equally over 24 hours, may even be below the substitution dosage. A constant cortisol level is thus obtained in place of the normal nyctohemeral rhythm (p. 300). Although more than two dozen actions of cortisol have been experimentally investigated on the whole organism, on organs, on cells, and on cell constituents (WEISSMANN, 1971), only the morphology of the anti-inflammatory process can be described; the mechanism of the anti-inflammatory action of glucocorticoids remains largely unexplained. Yet some insight into this mechanism is dawning, on the basis of molecular biology: there is evidence that cortisol has a stabilizing effect on the cellular membrane and on the cell organelles. It also protects the lysosomes in particular from being destroyed (WEISSMANN, 1964). Cortisone prevents the fusion of lysosomes, which is important for this system in intact cells and inhibits the release of lysosomal material into the damaged tissue (WEISSMANN, 1971). It can be morphologically demonstrated that exudative and proliferative inflammatory processes regress due to a decrease in the capillary permeability and a limitation of the fibroblastic proliferation (p. 305). The inflammatory hyperemia decreases. The action is probably achieved partly via inhibition of the release of kinins (p. 977). There is, however, no action on old granulation tissue. These actions are pharmacological ones, although in some situations the body can produce glucocorticoids in amounts with antiphlogistic effects. It is improbable that physiologically the mineralocorticoids favor the process of inflammation (p. 311).

The anti-inflammatory action of the steroids is connected with and parallels their gluconeogenetic activity and their inhibitory effect on the hypothalamus and the pituitary.

The effects obtained with cortisol can also be achieved with ACTH infusions or intramuscular injections of depot-ACTH. Apart from the inconvenience of administration, ACTH also has the disadvantage of causing the release of increased amounts of androgens by the adrenals, and in contrast to most of the cortisone derivatives it leads to sodium and water retention. In our opinion, ACTH has no therapeutic indications and its use is limited to diagnostic tests.

Inflammation can be useful to the organism, by inhibiting the spread of noxious agents and invalidating them. Often, however, the damage caused by the inflammation itself is considerably more detrimental to the organism than the original harm due to the noxious agents. Anti-inflammatory therapy is justified in such cases. The dramatic effect of cortisol and its derivatives is usually limited to the duration of administration. The glucocorticoids suppress the inflammatory processes but do not eliminate them. The inflammatory manifestations usually return unchanged when the drug is withdrawn. This is an important point in the indications for cortisone treatment. The main indication for cortisol and its derivatives is self-limiting inflammatory disease or inflammatory disease with an episodic character where severe permanent damage can be caused during the inflammatory phase. Acute rheumatic carditis is an example. Anti-inflammatory therapy with hormones is only of limited use in chronic inflammatory diseases, since the side effects can sometimes exceed the symptoms of the original disease. Even the most serious side effects must be accepted where there is a vital indication in potentially fatal diseases (panarteritis nodosa, pemphigus, exfoliative dermatitis, erythroderma, dermatomyositis). High doses should be given at the beginning of treatment and gradually reduced to the lowest dose which is still effective. In chronic diseases which result in invalidity but are not potentially fatal, it is advisable to give doses in an attempt to produce a palliative action sufficient to permit the patients to work again. The lowest possible dose is given at first and this is gradually increased until the symptoms are tolerable. Inflammatory diseases in which the action of the noxious agent regresses and the reaction of the host organism is the dominant feature are especially well suited for anti-inflammatory cortisone therapy (allergy, rheumatic diseases, collagen diseases). Not only does cortisol have a decisive effect in exudative inflammations, but certain granulomatous processes such as sarcoidosis or histiocytosis may also be considerably improved by its use.

Infections were previously considered an absolute contraindication for treatment with cortisone since the spread of the pathogens could be favored by the suppression of the inflammatory reaction. Cortisone therapy in combination with chemotherapy and antibiotics can now be useful or even the deciding factor in some infections with hyperergic inflammatory reactions, e.g. certain forms of tuberculosis (meningitis). Experience and the utmost care are essential when cortisone is used in the treatment of these infectious diseases.

b) Immunosuppression

Contrary to original views, the formation of antibodies is not suppressed by cortisol and its derivatives. Nevertheless, cortisol in high doses inhibits the immunological processes by

blocking the binding of the antibody with the antigen and in particular with intracellular antigens, i.e. invasion of the antigen-containing tissue by sensitized cells is prevented. Cortisol also influences the lymphocytes and plasma cells. It causes a delay in their formation and maturation, inhibits their release into the blood, and promotes lymphocytolysis. The inhibition of the activation of kinins during the antigen-antibody reaction can also influence immunological processes. It is often difficult to separate immunosuppression and inflammatory inhibition from each other, especially when both properties of cortisol come into effect in the autoimmune diseases. Thus, immunohematological disorders, especially the immunohematolytic syndromes and less commonly autoimmune leukopenia and autoimmune thrombocytopenia, are indications for treatment with cortisol. Autoimmune diseases such as visceral lupus erythematosus and panarteritis nodosa are important indications for cortisol and its derivatives, and combination with azathioprine might be indicated. It is not known to what extent the beneficial effect on nephrosis is due to immunosuppression. Immunosuppression with cortisol derivatives combined with azothioprime is now a routine procedure for organ transplantations.

Anti-inflammatory action and immunosuppression explain the anti-allergic properties of cortisol. Acute potentially fatal allergic reactions, such as anaphylactic shock and similar reactions, are an absolute indication for treatment with cortisol. The greatest caution is indicated in chronic allergic diseases because of the side effects. Local application of cortisol derivatives is justifiable in these conditions. The anti-inflammatory action of cortisol alone is probably responsible for the improvement in chronic inflammatory autoimmune and allergic disorders such as rheumatoid arthritis and chronic asthmoid bronchitis. The indications are relative in these disorders, because of the side effects with long-term treatment.

c) Oncological Indications

Oncological indications are advanced stages of metastasizing carcinomas of the breast and prostate. An improvement of the general state can be expected in particular. The action is not due to inhibition of the adrenals or "pharmacological adrenalectomy" as was previously supposed. It is probably due to the anti-inflammatory properties of cortisol and its derivatives. It is possible that cortisone interferes with the metabolism of ribonucleic acid (KUMMER, 1968). The glucocorticoids are indicated especially in the following complications: 1. Metastases in the central nervous system and in the liver. Doses of 50–100 mg prednisone daily are necessary. 2. Pleural and pericardial effusions due to the tumor. These are often resistent to other hormonal therapy, but regression can be achieved in more than 50% with an initial treatment with 50–100 mg daily. Prednisone should later be given sporadically with therapy-free intervals.

Treatment with cortisol is also indicated in acute leukosis when it should be used in combination with cytostatics. In this condition high doses of prednisone, up to 400 mg daily, must be given. The success obtained with this treatment is very impressive but lasts for a limited time. The younger the patient, the more lasting and better the result. In chronic lymphadenosis cortisol is indicated only in the presence of disturbing infiltrations, complicating anemia, or thrombocytopenia. Cortisol has also proved to be effective in combination with cytostatics and radiation for malignant lymphomas.

d) Hypocalcemic Effect

In *hypercalcemia* of various etiologies, particularly when it is associated with malignant tumors but not with hyperparathyroidism, the serum calcium falls or returns to normal under the influence of cortisol. This action can save a patient's life. The mode of action is not explained although cortisol inhibits calcium absorption from the intestine as well as reabsorption in the renal tubules. Cortisol also has a hypocalcemic effect in patients fed parenterally, however.

e) Other Indications

Paget's disease with arteriovenous shunts disturbing hemodynamics and alopecia areata are indications for treatment with cortisol, although there is still no explanation for the mode of action.

f) Untoward Side Effects of Pharmacological Treatment with Cortisone

The so-called side effects are inseparably bound up with the anti-inflammatory action of cortisone and its derivatives, with the one exception of sodium retention. The untoward side effects of treatment with cortisol are best recognized in the symptoms of Cushing's syndrome; as a rule treatment with over 50 mg cortisol or the corresponding equivalent doses of the derivatives for over 2 weeks results in an iatro-

genic Cushing's syndrome. The degree of the changes and the speed at which they develop are subject to wide individual variations.

Osteoporosis is the most severe side effect in long-term treatment. There is a correlation between the dose of cortisol and the duration of the treatment, but again there is such wide individual variation that some patients treated with average doses for over 10 years do not have clinically detectable osteoporosis, while other patients develop pathologic fractures after 2.5 years even with lower doses. The pathogenesis is only partly explained. It is related to the switchover of the metabolism from protein synthesis to carbohydrate production. It is also related to the actions of cortisol on calcium metabolism. Qualitatively and quantitatively inadequate matrix probably has some effect in this type of osteoporosis. Prophylaxis of cortisol-induced osteoporosis is as uncertain as its pathogenesis. Movement, pressure, and traction exerted by the muscular tone on the bones are the greatest stimuli for building bone and the best means of preventing osteoporosis. Very high doses of androgens and anabolic steroids, usually in the virilizing range, can convert a negative nitrogen balance into a positive one. The metabolic changes due to cortisone are not, however, abolished. It can be shown histologically that the effects of cortisone can be reduced but not abolished by testosterone. It is difficult to assess to what extent cortisone osteoporosis can be clinically diminished or delayed by anabolic drugs. It is generally accepted that anabolic medication is not certain to prevent cortisone osteoporosis. An optimal nutritional state is reached with additional calcium medication, but the organism absorbs less calcium when the endogenous supply of calcium rises, and it is questionable just how much can be achieved with increased intake. Apart from the cortisone osteoporosis, aseptic necroses have frequently been observed in the parts of the skeleton most exposed to pressure, such as the head of femur and humerus. Charcot's joints, with the disappearance of cartilage, have also been observed after local cortisol treatment. The anti-anabolic action of cortisol leads to a standstill in growth and maturation in children. This is reversible, however, after withdrawal of the cortisol.

The increase in gluconeogenesis caused by cortisone in the usual anti-inflammatory dosage results in an increase of 10–20 mg% in the blood sugar in healthy subjects. The increased amounts of glucose produced are deposited as glycogen and fat under the influence of increased insulin secretion. Cortisol causes potential diabetes to become subclinical or manifest only when the insulin reserve of the pancreas is inadequate (so-called "steroid diabetes"). One should not refrain from giving cortisol to the diabetic if there is a definite indication, but the increased need for insulin must be met. The effects on the psyche again vary widely individually and are dependent on the premorbid personality. They vary from mild euphoria or depression to all degrees of schizophrenia like symptoms and the "acute exogenous reaction type". Finally, the disfiguring changes in the face, in the body stature, and in the skin, with rubeosis, acne, and hirsutism can be a severe psychological burden, particularly to female patients. Peptic ulcer is a side effect of cortisol which curiously does not occur in Cushing's syndrome. Cortisol does not promote the secretion of hydrochloric acid or of pesin but the incidence of both gastric and duodenal peptic ulcers is increased during cortisone therapy. This is probably attributable to influence on the production of the protective mucin by the gastric mucosa.

Other untoward side effects are the increased risk of infection, acute pancreatitis and pancreatic necrosis, influence on the nervous system, intracranial pressure leading to the so-called "pseudotumor cerebri", changes in dentine and dental enamel, changes in the eye, cataract of the subcapsular region of the lens, increased intraocular pressure leading to glaucoma, and after local application trophic corneal disorders (for literature see KAISER, 1973).

Secondary adrenocortical insufficiency is an unavoidable result of effective long-term cortisol therapy. An adrenal insufficiency lasting for several days is always seen after treatment with over 60–75 mg of cortisol for more than one week, or after 15 mg prednisone daily in 3–4 doses. First the adrenals secrete only the basal amounts of cortisol and later they become less responsive to ACTH, i.e. there is first an inhibition of the hypothalamus, followed by changes in the adrenal cortex itself. The response usually returns in 1–2 days. The adrenocortical secretion does not exceed the basal values, however, since the pituitary has no ACTH reserve. A maximal adrenal atrophy is reached in 15–20 weeks with a dose of 60 mg or more of cortisol. This state often lasts up to 6 months. Stress is dangerous for the patients during this period. There is secondary adrenal insufficiency, and there have been fatalities. The degree and duration of the secondary adrenal insufficiency is dependent to some extent on the duration and dosage of the steroid medication and possibly also on the particular compound when amounts high enough to exert an anti-inflammatory effect are given, but individual factors play a very large part. The

localization of the disturbance does not seem to be the same in all patients. In many patients, there is a temporary phase of hyporeactivity of the adrenal cortex (GRABER, 1965), and the rise in the ACTH concentration in the plasma sometimes precedes that of the plasma corticoids by a few months. In other cases, a disturbance in ACTH secretion is dominant (CARREON, 1960). Cases are also known in which no change in adrenal function can be detected after years of treatment with high doses of steroids (JASANI, 1967). An intravenous injection of cortisol hemisuccinate should always be kept ready during any operation on a patient who has received corticosteroids up to 6 months previously. A "preventive steroid protection" is not advisable in general, since only 5–10% of patients treated with cortisone react inadequately to surgical stress. The secondary adrenal insufficiency can present as tiredness, anorexia, malaise and tendency to collapse. Adrenal atrophy does not occur after ACTH therapy, but the ACTH reserve in the pituitary is diminished. Incidents, however, seem to occur rather less frequently. To prevent secondary adrenal insufficiency it is advisable to reduce the cortisol or its derivatives step by step, by withdrawing first the nightly dose and then the evening dose, finally giving a dose in the morning only. This procedure re-establishes the daily rhythm of cortisol secretion. Depot-ACTH given intramuscularly for 3–5 days after the withdrawal of cortisone restores the capacity of the adrenals to respond, but little is actually gained since ACTH secretion by the pituitary is inhibited.

It has recently been recommended that high pharmacological doses of cortisone be given only once daily in the morning, or a synthetic glucocorticoid with a long half-life intermittently every other day in a single dose in the morning (DUBOIS, 1963; HARTER, 1963; GRANT, 1965; MACGREGOR, 1969; MARTIN, 1969), or that steroids be administered on three consecutive days of one week. The ACTH secretion should start again after 12 or 36 hours and adrenal atrophy is thought to be prevented by these methods of administration. The pharmacological action is thought to be adequate during this time when high doses are used. The experience obtained so far with this intermittent steroid therapy varies, however, and no definite conclusion can yet be drawn as to whether the risk of a secondary adrenal insufficiency can in fact be considerably reduced without diminishing the therapeutic action of the steroid compound at the same time. Monitoring by means of the insulin plasma cortisol test and the methyrapone test has also revealed disorders in the hypothalamo-pituitary-adrenal system after *intermittent* steroid treatment (MARTIN, 1968; MALONE, 1970).

Secondary adrenal insufficiency after long-term cortisone therapy is best avoided or reduced by gradual reduction of the dose of cortisone by 25–10 mg weekly or of the cortisone derivatives by the corresponding amounts.

A second withdrawal syndrome worth mentioning is the flare-up of the inflammatory manifestations. This occurs particularly frequently in rheumatoid arthritis. Mild exacerbations disappear after a few days. In more severe instances, the cortisone dosage must be increased again, and an attempt made to reduce it even more slowly, possibly assisted by other antirheumatic agents.

A large number of cortisol derivatives have been examined for advantages over the genuine hormone cortisol in pharmacological use. The first major breakthrough was achieved with the artificial steroids prednisone and prednisolone, which have a greatly reduced sodium-retaining effect as compared to cortisol, due to a double bond 1–2 at the A ring. Further modifications such as methylation and fluoridation at atoms 9, 6 and 16 have produced more potent preparations per unit of weight but not improved the ratio of desired to undesired effects. Thus, prednisone is currently the most economical derivative of cortisol and therefore the standard drug for anti-inflammatory treatment. The anti-inflammatory action has never successfully been separated from the gluconeogenetic and catabolic actions although the hypothalamic side effects and the peripheral metabolic effects have been successfully dissociated. The preparation 6-dehydro-16 methyl cortisol appears to have an inhibitory effect on the hypothalamus without having any essential peripheral metabolic actions. It is of therapeutic interest as a therapeutic agent or at least as a palliative drug in Cushing's syndrome.

The preparations triamcinolone, prednisolone, dexamethasone, betamethasone and paramethasone have basically the same qualitative actions as prednisone except for the mineralocorticoid effect. Their half-lives are longer and may very well be related to the varying binding to the carrier proteins in the plasma.

An exception within the group of cortisol derivatives, is the preparation 9-alpha-fluorocortisol, which has a very pronounced mineralocorticoid action in addition to its marked glucocorticoid effect. It is well absorbed orally and replaces aldosterone for substitution in primary adrenal insufficiency. In a dosage of 0.1–0.3 mg daily it can be useful in the treatment

of various hypotensive states and especially of orthostatic hypotension (HICKLER, 1959; LEGELER, 1971).

g) Forms of Administration

Oral administration of cortisol derivatives in tablet form is the route of choice. A continuous intravenous infusion of cortisol or of prednisolone hemisuccinate or phthalate is given during operations. In emergency these preparations can also be administered intramuscularly, but the onset of action is slower. Local external application on the skin and mucosa is also possible and advantageous (equivalent dosage schedule is given on p. 1073).

h) Indications for Treatment with Cortisone

Cortisone is a potent pharmacological agent, but the drawbacks must never be overlooked. Prophylaxis of the damage caused by cortisone is hardly possible and the risk of untoward side effects can never be eliminated completely. Thus the doctor must be aware of his responsibility and weigh the danger of the condition to be treated carefully against that of the therapy. In vital indications or in disabling diseases, e.g., pemphigus, lupus erythematosus and panarteritis nodosa, the risk of osteoporosis has to be accepted. Even if osteoporosis becomes manifest, it is sometimes correct to continue cortisone therapy. On the other hand, the decision to start cortisone treatment for a skin condition which is troublesome but not potentially fatal entails severe consequences as the treatment may be long-term. The following can be taken as a guiding rule: treatment in short bouts of a few days or at the most weeks is permissible in diseases where only the subjective symptoms are severe, but long-term treatment or sporadic treatment which might extend into a long-term therapy is only warranted if there is a vital indication. Doctor and patient must both be aware of the risks and be prepared to accept them. The decision as to where to draw the line must be made individually for each case.

2. Anabolic Therapy

a) Mode of Action

In addition to its effects on the secondary sexual characteristics of the male and a virilizing action on the women, the male sex hormone, testosterone, promotes protein synthesis in the liver and periphery in both males and females. It promotes growth, but as it stimulates skeletal maturation at the same time, growth comes to a premature standstill after a temporary acceleration (Chap. XIX, p. 1032).

Steroid compounds, mainly derivatives of testosterone, which promote protein synthesis without the virilizing properties of testosterone, or with less of these properties, have been under investigation for the past 20 years. A whole series of derivatives of testosterone or progesterone with an improved ratio of protein synthesis-promoting action, the so-called anabolic effect, to androgenic action have been successfully produced. Many anabolic agents are now commercially available (KRÜSKEMPER,

Table 4. The most important indications for cortisone and its derivatives (in parenthesis: "only in special conditions")

Allergic diseases
Anaphylactic shock
Serum sickness
Status asthmaticus (asthma bronchiale)
Dangerous insect bites
Urticaria
Angioneurotic edema
Rheumatic and other inflammatory tissue diseases
Acute rheumatic fever with carditis
(Rheumatoid arthritis)
Lupus erythematosus disseminatus
Periarteriitis nodosa
Skin diseases
Pemphigus
Exfoliative erythroderma
Dermatomyositis
(Certain allergic dermatitides)
Blood diseases
Acquired hemolytic anemia
Idiopathic thrombopenia
(Leukemia)
(Lymphogranuloma)
Gastrointestinal diseases
Colitis ulcerosa
Enteritis regionalis
Sprue
(Hepatitis)
Kidney diseases
(Nephrosis)
Infectious diseases in combination with antibiotics
Meningitis tuberculosa
Lung diseases
Sarcoidosis and other granulomatoses
(Tuberculosis)
Eye diseases
Certain allergic and inflammatory eye diseases
Organ transplantations
For immunosuppressive therapy in combination with azothiaprine
Malignancy
For symptomatic palliative treatment, especially hypercalcemic syndrome

Table 5. Generic names and equivalent dosages of the most-used anti-inflammatory steroids

Cortisone	25 mg
Cortisol (Hydrocortisone)	20 mg
Prednisone	5 mg
Prednisolone	5 mg
16-Methylen-prednisolone	10 mg
6α-Methyl-prednisolone	4 mg
Triamcinolone	4 mg
Paramethasone	2 mg
Dexamethasone	0.75 mg
Betamethasone	0.75 mg
Water-soluble preparations:	
Cortisol hemisuccinate	
Paramethasone-disodium phosphate	
Dexamethasone phosphate	
Prednisolone hemisuccinate	
Prednisolone-Na tetrahydrophthalate	
Methyl-prednisolone hemisuccinate	

1965). Complete elimination of the androgenic action has never been achieved. Great caution is necessary in extrapolating conclusions drawn from their actions in animals to man since it is not possible to predict how a compound which has acted favorably in animals will affect the human. In addition, the sensitivity to the virilizing properties in the anabolics varies widely from one woman to another, so that it is only possible to give tentative estimates of borderline doses with which there is generally no risk of virilization. Children, premature babies, and the fetus are especially sensitive to anabolic drugs, which are therefore contraindicated in pregnancy.

Anabolic agents are recommended in chronic tiredness, to improve the general condition and to increase the appetite and weight, particularly in chronic illness and in advanced age. They have been credited with shortening convalescence after operations and after fractures and also with shortening the duration of acute and chronic infections. It has also been claimed that they improve the condition in cachectic disease, improve osteoporosis, reduce the catabolic action during long-term cortisone therapy, and improve delayed growth and retarded weight gain in children.

The indications, however, are generally much too liberal. Anabolic agents cannot increase the protein synthesis beyond given limits when protein intake is inadequate or proteins are lost, i.e. in amino-acid deficiency. It has been demonstrated that a negative nitrogen balance can become positive under the influence of anabolic drugs only when there is a constant low protein intake. Unlimited protein intake can achieve the same results. Protein synthesis is regulated by physical activity, nutrition, and different hormones (growth hormone, insulin). Adequate nutrition is, however, a primary requirement. Anabolics should therefore only be employed when simpler and less dangerous measures have failed.

Until recently the only indication based on confirmed facts was thought to be postmenopausal and senile osteoporosis. However, there is currently some controversy about the pathogenesis of osteoporosis again, and the value of anabolic agents is being questioned for reasons based on histometric measurements in vitally stained series of bone biopsies. The hypothesis of increased skeletal breakdown rather than inadequate osteoblastic activity is currently prevalent. Nevertheless, subjective improvement is achieved with anabolic agents in osteoporosis, possibly by an effect on the muscles, and anabolics are still indicated along with other measures in crippling osteoporosis, despite the uncertain theoretical bases.

The use of anabolic agents to promote growth in dwarfism or in delayed growth, and the great caution necessary with this treatment, which should only be undertaken by the very experienced doctor, are discussed in Chap. XIX.

A nitrogen deficit and calcium loss after multiple or severe fractures with prolonged immobilization is usually best treated with an adequate intake of proteins, calories, and calcium, and exercises even for patients confined to bed. Mobilization as quickly as possible with auxiliary measures is the most effective method. Nevertheless, the use of anabolic agents in addition is occasionally justified. There is no evidence that anabolics, including testosterone, can shorten the surgical or medical convalescent period, but there are no controlled prospective studies. It is stated that most anabolic drugs effectively improve anorexia, tiredness, and other subjective symptoms. Only a few controlled comparative studies are available (WATSON, 1959). The appetite does appear to be improved in some patients, and there has been a short trial of anabolic agents as appetite stimulants in adults in whom other drugs have failed.

It must be borne in mind when anabolic drugs are given to geriatric patients that androgens raise cholesterol and beta-globulins, which promote coronary sclerosis.

Convalescence is not a general indication for anabolics. True convalescence has a rapid course and cannot be accelerated. Delayed convalescence is usually due to a second unknown disease, and loss of appetite due to psychological factors, such as anorexia nervosa, is not affected by anabolics.

Preparations mixed with vitamins are especially questionable since they are offered as roborants and the character of the anabolic substance is hidden. "General exhaustion", "school tiredness", and "spring tiredness" should be examined for underlying causes and are not indications for hormone therapy. There are less harmful drugs which will have an equally good placebo effect.

The use of anabolic drugs in renal failure to reduce the urea by protein synthesis has no clinical (SCHWARTZ, 1968) or experimental (GERBER, 1961) basis. On the contrary, they should be used with the utmost caution in renal diseases because of the sodium retention and the formation of edema.

Whether there is a causal relationship between long-term anabolic steroid therapy and development of hepatocellular carcinoma has still to be proved true or false (JOHNSON, 1972).

b) Indications and Contraindications

All anabolic androgens methylated at the atom 17 have a more or less cholestatic action on the liver. With the less harmful of these compounds only a temporary increase in the retention of bromsulphthalein is demonstrable. These effects on the liver are generally reversible. However, fatalities due to hepatic failure during the use of some anabolic steroids have been described.

Virilization is the most frequent side effect; it is not dangerous but is a burden to women. It is usually irreversible even after the anabolic treatment has been discontinued. The maximum nonvirilizing doses for long-term treatment are: for metandrostenolone (Dianabol) 5 mg daily, for nandrolone decanoate (Decadurabolin) 50 mg i.m. every three weeks, for methenolone nantate (Primobolan depot) 50–100 mg i.m. every 2–3 weeks depending on the body weight. For oral methenolone acetate (Primobolan) the maximum dose is 10–20 mg daily. Damage can be avoided with these doses even in hypersensitive women, but mild side effects are nevertheless still possible. Changes in the voice arise particularly frequently even with correct doses in sensitive women. The voice can change within days. Laryngeal growth is not necessary for this. Tissue changes in the vocal cords develop under the influence of androgens, resulting in an altered vibration. The voice becomes hoarse, breaks, and finally becomes a chest voice. The changes in the voice are irreversible.

The increase in the libido due to the action of androgens varies considerably. In some cases it is only stimulation while in others it can be very distressing. Hirsutism, acne, amenorrhea, and growth of the clitoris are also possible with doses within these limits in sensitive women, even though they do not generally arise with the recommended dosages.

The damage caused by extensively used anabolic agents is probably greater than the benefit gained. Aplastic anemias, myelofibrosis, and myelid metaplasia are further indications for testosterone or anabolic agents, as they promote erythropoiesis. The side effects, in particular the virilization, must be accepted in these serious diseases, and testosterone in high or in very high doses is indicated for both men and women.

3. Progesterone in Respiratory Dysfunction

Progesterone increases alveolar ventilation in pregnancy and in the luteal phase of the cycle, and also in men when given in pharmacological doses. It has been used in high doses in obstructive pulmonary emphysema (TYLER, 1960) and also in hypoventilation of severe obesity (Pickwick syndrome). Ventilation can be improved with 100 mg progesterone daily i.m.; the hypercapnia is normalized and the respiratory acidosis disappears (LYONS, 1968).

It is still undecided whether treatment with progesterone has a favorable effect on scleroderma (KORTING, 1967).

4. Glucagon

a) In Heart Failure and Arrhythmias

In high pharmacological doses glucagon has an effect on the circulatory system. There is a slight positive chronotropic effect on the sinus rhythm and a positive inotropic effect on the myocardium, and the cardiac output is increased. It accelerates auriculoventricular conduction in the heart and slightly decreases the peripheral resistance.

These effects on the heart are not dependent on catecholamines and are not influenced by beta-blocking agents. The positive inotropic effect is independent of and additive to that of digitalis. It activates adenylcyclase, so supplying ATP, and has a glycogenolytic effect on the liver via cyclic AMP.

The dosage is 5 mg i.v. followed by an i.v. infusion of 2 mg/h in 5% glucose solution over some hours or days for the following complications: heart failure in acute arrhythmias or metabolic disorders where catecholamines or digitalis are contraindicated. Intoxication by digitalis or beta-blocking agents is said to be an absolute indication. Side effects are nausea and vomiting, while glycemia and electrolytes are not significantly changed. So far, little or

no influence of glucagon on chronic heart failure has been proved.

This kind of cardiac treatment is still in the experimental phase; it involves high costs and the results so far are encouraging but not conclusive. There are some misgivings associated with the fact that *in vitro* the positive inotropic effect can only be demonstrated in the healthy and not in the damaged papillary muscle (EPSTEIN, 1970).

Table 6. Indications for glucagon in circulatory disorders. (After ROBERT and HUMAIR, 1970)

Generally, it has to be regarded as an adjunct to standard therapeutic measures or for use when these are contra-indicated

Acute heart failure, cardiogenic shock or congestive heart failure complicated by
1. *Dysrhythmias*
Bradycardia, e.g. auricular fibrillation
Atrioventricular block
Ventricular extrasystoles, increased ventricular automatism
2. *Metabolic disorders*
Hypokalemia
Hypoxia
Renal failure
3. *Drug intoxications*
Beta-blocking agents
Antiarrhythmic agents
Digitalis

b) In Paget's Disease

Glucagon given in i.v. infusions (0.2–0.8 mg/h) seems to have a beneficial effect on bone pains as well as on the metabolic parameters of the disease. There is a fall in plasma alkaline phosphatase and in urinary calcium and hydroxyproline excretion. The mechanism of this action is not yet known. Release of calcitonin or pyrophosphatases might be involved (CORDON, 1971).

References

Endocrine Therapy of Carcinomas of the Breast, Prostate and Corpus Uteri and of Prostatic Hypertrophy

The Following Works Incorporate Detailed Lists of References

GELLER, J., BORA, R., ROBERTS, T., NEWMAN, H., LIN, A., SILVA, R.: Treatment of benign prostatic hypertrophy with Hydroxyprogesterone Caproate. J. Amer. med. Ass. **193**, 115 (1965).

GRIEM, M. L., JENSEN, E. V., ULTMANN, J. E., WISSLER, R. W.: Breast cancer: A challenging problem. Rec. Res. Cancer Research, No. 42. Berlin-Heidelberg-New York: Springer 1973.

MARBERGER, H., MADERSBACHER, H.: Hormontherapie beim Prostatacarcinom. Verh. dtsch. Ges. inn. Med. **73**, 518 (1967).

MARTZ, G.: Die hormonale Therapie maligner Tumoren. Heidelberger Taschenbücher No. 41. Berlin-New York-Heidelberg: Springer 1968.

NOWAKOWSKI, H.: Möglichkeiten und Grenzen der endokrinen Therapie des metastasierenden Mammacarcinoms. Verh. dtsch. Ges. inn. Med. **73**, 505 (1967).

SPRATT, J. S., DONRGAN, W. L.: Cancer of the breast. Philadelphia-London: Saunders 1967.

TAUSK, M.: Pharmakologie der Hormone. Stuttgart: Thieme 1970.

VERNET, D.: Les gestagènes: voie nouvelle de l'hormonothérapie dans l'hypertrophie bénigne de la prostate. Praxis **59**, 1708 (1970).

Pharmacological Use of Hormones

Cortisol and its Derivatives

ACKERMANN, G. L., NOLAN, CH. M.: Adrenocortical responsiveness after alternate-day corticosteroid therapy. New Engl. J. Med. **278**, 405 (1968).

ALBRIGHT, F., HENNEMAN, P. H.: Suppression of Paget's disease with ACTH and cortisone. Trans. Ass. Amer. Phycns **88**, 238 (1955).

ARNOLDSON, H.: Pituitary-adrenocortical function after multennial steroid therapy: significance of dosage and responsiveness to stress. In: An introduction to clinical neuroendocrinology, p. 201. Basle-New York: S. Karger 1967.

BACON, P. A., MYLES, A. G., BLARDWELL, C. G., DALY, J. R.: Corticosteroid withdrawal in rheumatoid arthritis. Lancet **1966 II**, 935.

BEGEMANN, H., KABOTH, W.: Nebenwirkungen der Cortisonderivate. Internist (Berl.) **8**, 85 (1967).

BETHGE, H.: Die steroidinduzierte Nebennierenrindenunterfunktion. Pathogenese, Klinik, Diagnostik, Prophylaxe und Therapie. Klin. Wschr. **48**, 317 (1970).

BOLAND, E. W.: Clinical comparison of the newer anti-inflammatory corticosteroids. Ann. rheum. Dis. **21**, 176 (1962).

BRUNNER, K., KAISER, G., MARTZ, G.: Die Corticosteroidtherapie der chronischen lymphatischen Leukämie. Dtsch. med. Wschr. **88**, 1128 (1963).

CARREON, G. G., CANARY, J. J., MEYER, R. J., KYLE, L. H.: Adrenocortical function after long-term corticoid therapy. J. Lab. clin. Med. **56**, 235 (1960).

CARTER, M. E., JAMES, V. H. T.: Pituitary-adrenal, response to surgical stress in patients receiving corticotrophin treatment. Lancet **1970 I**, 328.

DUBOIS, E. L., ADLER, D. C.: Single daily dose oral administration of corticosteroids in theumatic disorders: an analysis of its advantages, efficacy, and side effects. Curr. ther. Res. **5**, 43 (1963).

Editorial: The hypothalamic-pituitary axis. New Engl. J. Med. **279**, 319 (1968).

FRUHMANN, G.: Cortisonderivate bei allergischen Krankheiten. Internist (Berl.) **8**, 111 (1967).

GRABER, A. L., NEY, R. L., NICHOLSON, W. E., ISLAND, D. P., LIDDLE, G. W.: Natural history of pituitary-adrenal recovery following long-term suppression with corticosteroids. J. clin. Endocr. **25**, 11 (1965).

GRANT, S. D., FORSHAM, P. H., DI RAIMONDO, V. C.: Suppression of 17-hydroxycorticosteroids in plasma and urine by single and divided doses of triamcinolone. New Engl. J. Med. **273**, 1115 (1965).

HARTER, J. G., REDDY, W. J., THORN, G. W.: Studies on intermittent corticosteroid dosage regimen. New Engl. J. Med. **269**, 591 (1963).

HICKLER, R. B., THOMPSON, G. R., FOX, L. M., HAMLIN, J. T.: Successful treatment of orthostatic hypotension with 9-α-Fluorohydrocortisone. New Engl. J. Med. **261**, 788 (1959).

JASANI, M. K., BOYLE, J. A., GREIG, W. R., DALAKOS, T. G., BROWNING, M. C. K., THOMPSON, A., BUCHANAN, W. W.: Corticosteroid-induced suppression of the hypothalamo-pituitary-adrenal axis: observations on patients given oral corticosteroids for rheumatoid arthritis. Quat. J. med. **36**, 261 (1967).

KAISER, H.: Cortisonderivate in Klinik und Praxis, 6. Aufl. Stuttgart: Thieme 1973.

KUEMMERLE, H. P., SENN, A., RENTCHNICK, P., GOOSSENS, N.: Klinik und Therapie der Nebenwirkungen. Stuttgart: Thieme 1960.

KUMMER, D., OCHS, H. D.: Cytostatischer Wirkungsmechanismus von Cortisol und verwandten Steroiden. Z. ges. exp. Med. **147**, 291 (1968).

LABHART, A.: Vermeidbare und nicht vermeidbare Schäden der Hormontherapie. Cortisonosteoporose und virilisierende Wirkung der anabolen Steroide. Schweiz. med. Wschr. **96**, 807 (1966).

— MÜLLER, J., MAYOR, G.: Der chirurgische Eingriff unter hochdosierter Cortison-Therapie, bei Cushing-Syndrom und bei primärer oder sekundärer Nebennierenrinden-Insuffizienz. In: Chirurgische Operationslehre. Founded by BREITNER, ZUKSCHWERDT und KRAUS, Eds. Munich-Berlin-Vienna: Urban & Schwarzenberg 1969.

LANDOLT, A. M., SIEGFRIED, J.: Zur Behandlung maligner, metastasierender Tumoren mit der stereotaktischen transsphenoidalen Elektrokoagulation der Hypophyse. Schweiz. med. Wschr. **100**, 1297 (1970).

LEGELER, H. J., STOLL, K. D.: Bericht über die klinische Prüfung von 9α-Fluorhydrocortison zur Behandlung hypotoner und orthostatischer Kreislaufregulationsstörungen. Arzneim.- Forsch. (Drug Res.) **21**, 1144 (1971).

LEVELL, M. J., STITCH, S. R., NORONHA, M. J.: Impairment of the pituitary-adrenal axis following prolonged therapy with corticotrophin. Acta endocr. (Kbh.) 65, 608 (1970).

LIDDLE, G. W., FOX, M.: Structure-function relationships of anti-inflammatory steroids. In: Inflammation and diseases of connective tissue: a Hahnemann symposium, L. C. MILLS and J. H. MOYER, Eds. Philadelphia, London: Saunders 1961.

MACGREGOR, R. R., SHEAGREN, J. N., LIPSETT, M. B., WOLFF, S. M.: Alternate-day prednisone therapy. Evaluation of delayed hypersensitivity responses, control of disease and steroid side effects. New Engl. J. Med. **280**, 1427 (1969).

MAGNENAT, G., CRUCHAUD, S., RUEDI, B.: Incidences hypophyso-serrénaliennes de la corticothérapie prolongée à faibles doses chez les asthmatiques. Schweiz. med. Wschr. **102**, 804 (1972).

MALONE, D. N. S., GRANT, I. W. B., PERCY-ROBB, I. W.: Hypothalamo-pituitary-adrenal function in asthmatic patients receiving long-term corticosteroid therapy. Lancet **1970 II**, 733.

MARTIN, M. M., GABOARDIE, F., PODOLSKI, S., RAITI, S., CALCAGNO, P. L.: Intermittent steroid therapy. Its effect on hypothalamic-pituitary-adrenal function and the response of plasma growth hormone and insulin to stimulation. New Engl. J. Med. **279**, 273 (1968).

NELSON, J. K., MACKAY, J. S., SHERIDAN, B., WEAVER, J. A.: Intermittent therapy with corticotrophin. Lancet **1966 II**, 78.

NICHOLS, T., NUGENT, C. A., TYLER, F. H.: Diurnal variation in suppression of adrenal function by glucocorticoids. J. clin. Endocr. **25**, 343 (1965).

RAPPAPORT, E., KUIDA, H., DEXTER, L., HENNEMANN, PH. H., ALBRIGHT, F.: The cardiac output in Paget's disease before and after treatment with cortisone. Amer. J. Med. **22**, 252 (1957).

THORN, G. W.: Clinical considerations in the use of corticosteroids. New Engl. J. Med. **274**, 775 (1966).

— JENKINS, D., LAIDLAW, J. C., GOETZ, F. C., DINGMAN, J. F., ARONS, W. L., STREETEN, D. H. O., MCCRACKEN, B. H.: Pharmacological aspects of adrenocortical steriods and ACTH in man. New Engl. J. Med. **248**, 232, 284, 323, 369, 414, 588, 632 (1953).

VORLÄNDER, K. O.: Cortisontherapie bei Auto-Immunkrankheiten. Internist (Berl.) **8**, 94 (1967).

WEISSMANN, G.: The effects of corticosteroids, especially on lysosomes and biomembranes. In: Rheumatoid arthritis; pathogenetic mechanisms and consequences in therapeutics, p. 577. W. MÜLLER, H.-G. HARWERTH, K. FEHR, Eds. London: Academic press 1971 (Colloquia Geigy).

WILBRANDT, W.: Über die Wirkung von Corticosteroiden und anderen entzündungshemmenden Stoffen. Schwz. med. Wschr. **96**, 1136 (1966).

Anabolic Steroids

ALBRIGHT, F., REIFENSTEIN, E. C., JR.: Parathyroid glands and metabolic bone disease. Baltimore: Williams & Wilkins Co. 1948.

ANDERES, A.: Eine Übersicht über die therapeutisch verwendeten anabolen Steroide unter besonderer Berücksichtigung ihrer chemischen Struktur. Praxis **59**, 650 (1970).

BIERICH, J. R.: Die klinische Anwendung anabolischer Steroide. Mschr. Kinderheilk. **114**, 444 (1966).

BREUER, H., KRÜSKEMPER, H. L., BARTSCH, W.: Klinisch-experimentelle Untersuchungen mit eiweißanabol-wirksamen 1-Methylandrostenolonen. Dtsch. med. Wschr. **87**, 1349 (1962).

DAMSTÉ, P. H.: Virilization of the voice due to anabolic steroids. Folia phoniat. (Basel) **16**, 10 (1964).

EDGREN, R. A.: A comparative study of the anabolic and androgenic effects of various steroids. Acta endocr. (Kbh.) Suppl. 87 zu **44** (1963).

Editorial: Androgenic and anabolic steroids. Brit. med. J. **1964 I**, 165.

FRUEHAN, A. E., FRAWLEY, TH. F.: Current status of anabolic steroids. J. Amer. med. Ass. **184**, 527 (1963).

GARDNER, F. H., NATHAN, D. G.: Androgens and erythropoiesis. III. Further evaluation of testosterone treatment of myelofibrosis. New Engl. J. Med. **274**, 420 (1966).

GERBER, M. W.: Zur Wirkung anaboler Hormone bei Azotämie. Helv. med. Acta **28**, 197 (1961).

GEYER, G., JESSERER, H.: Kann der gesteigerte Eiweiß-Katabolismus während einer Cortisontherapie durch anabole Steroide ausgeglichen werden? 7. Symp. Dtsch. Ges. Endokr. Berlin-Göttingen-Heidelberg: Springer 1961.

HOLZKNECHT, F.: Gefahren der Therapie mit Anabolica. Med. Welt **39**, 2238 (1965).

JOHNSON, F. L., FEAGLER, J. R., LERNER, K., MAJERUS, P. W., SIEGEL, M., HARTMANN, J. R., THOMAS, E. D.: Association of androgenic-anabolic steroid therapy with development of hepatocellular carcinoma. Lancet **1972 II**, 1273.

KORY, R. C., WATSON, R. N., BRADLEY, M. H., PETERS, B. J.: A six month evaluation of an anabolic drug, norethandrolone, in chronically underweight individuals. J. clin. Invest. **36**, 907 (1957).

KRÜSKEMPER, H. L.: Anabole Steroide, 2. Aufl. Stuttgart: Thieme 1965.

LABHART, A.: Vermeidbare und nicht vermeidbare Schäden der Hormontherapie. Cortisonosteoporose und virilisierende Wirkung der anabolen Steroide. Schweiz. med. Wschr. **96**, 807 (1966).

MARQUARDT, G. H., FISHER, CH. I., LEVY, P., DOROBEN, R. M.: Effects of anabolic steroids on liver function tests and creatine excretion. J. Amer. med. Ass. **175**, 851 (1961).

The Medical Letter: The anabolic-androgenic steroids, vol. 3, 41 (1961).

MÜLLER, A.: Médications anabolisantes et voix féminines. Pract. oto-rhino-laryng. (Basel) **26**, 91 (1964).

NOWAKOWSKI, H.: Klinische Indikationen der anabolen Steroide. Mkurse ärztl. Frtbild. **16**, 229 (1966).
OVERBEEK, G.A.: Anabole Steroide. Berlin-Heidelberg-New York: Springer 1966.
PRADER, A., ILLIG, R.: Use of anabolic agents in disorders of growth. In: Protein metabolism, an international symposium. Berlin-Göttingen-Heidelberg: Springer 1962.
— — Die Behandlung von Wachstumsstörungen mit anabolen Steroiden und mit Wachstumshormon. Dtsch. med. J. **13**, 547 (1962).
QUERIDO, A., KASSENAAR, A.A.: Nitrogen retaining steroids and their application in disease. Advanc. Metabol. disorders. **2**, 79 (1965).
REIFENSTEIN, E.C., JR.: The rationale for the use of anabolic steroids in controlling the adverse effects of corticoid hormones upon protein and osseous tissues. Sth. med. J. (Bgham, Ala.) **49**, 933 (1956).
— Control of corticoid-induced protein depletion and osteoporosis by anabolic steroid therapy. Metabolism **7**, 78 (1958).
SAARNE, A., BJERSTAF, L., EKMAN, B.: Studies on the nitrogen balance in the human during long-term treatment with different anabolic agents under strictly standardized conditions. Acta med. scand. **177**, 199 (1965).
SCHAFFNER, F., POPPER, H., CHESROW, E.: Cholestasis produced by the administration of Norethandrolone. Amer. J. Med. **26**, 249 (1959).
SCHÜPBACH, A.: Endokrines System und Skelett. Helvet. med. Acta **15**, 537 (1948).
SCHWARTZ, W.B., KASSIRER, J.P.: Medical management of chronic renal failure. Amer. J. Med. **44**, 786 (1968).
WATSON, R.N., BRADLEY, M.H., CALLAHAN, R., PETERS, B.J., KORY, R.S.: A six-month evaluation of an anabolic drug, norethandrolone, in underweight persons. Amer. J. Med. **26**, 238 (1959).
WYNN, V., LANDON, J., KAWERAU, E.: Studies of hepatic function during methandienon therapy. Lancet **1961I**, 69.

Progesterone in Respiratory Dysfunction

KORTING, G.W., HOLZMANN, H.: Gestagen-Behandlung der Sklerodermie. Aesthet. Med. **16**, 10, 291 (1967).
LYONS, H.A., HUANG, CH.T.: Therapeutic use of progesteron in alveolar hypoventilation associated with obesity. Amer. J. Med. **44**, 1881 (1968).
TYLER, J.M.: The effect of progesteron on the respiration of patients with emphysema and hypercarbia. J. clin. Invest. **39**, 34 (1960).

Glucagon in Heart Failure and Arrhythmias

BROGAN, E., KOZONTI, M.C., OVERY, D.C.: Glucagon therapy in heart failure. Lancet **1969 I**, 482.
CONDON, J.R.: Glucagon in the treatment of Paget's disease of bone. Brit. med. J. **1971 (4)**, 719.
EPSTEIN, S.E., SKELTON, C.L., LEVEY, G.S., ENTMAN, M.: Adenyl cyclase and myocardial contractility. Ann. intern. Med. **72**, 561 (1970).
GREENBERG, B.H., TSAKIRIS, A.G., MOFFIT, E.A., FRYE, R.: Hemodynamics and metabolic effects of glucagon in patients with valvular heart disease. Amer. J. Cardiol. **23**, 116 (1969).
PARMLEY, W.W.: The role of glucagon in cardiac therapy. New Engl. J. Med. **285**, 801 (1971).
— GLICK, G., SONNENBLICK, E.H.: Cardiovascular effects of glucagon in man. New Engl. J. Med. **279**, 11 (1968).
RATTI, R., ROTHLIN, M., SENNING, A.: Wirkung von Glukagon auf die Hämodynamik in der Frühphase nach Herzoperation. Schweiz. med. Wschr. **100**, 2171 (1970).
ROBERT, M., HUMAIR, L.: Effet cardiotonique du glucagon. Etude clinique et indications thérapeutiques. Schwz. med. Wschr. **100**, 1345 (1970).
SIMON, H., ESSER, H., FRICKE, G., BERCHTOLD, P., WARDACK, A., FRANKE, F.: Die Wirkung von Glukagon auf das insuffiziente Myokard. Verh. dtsch. Ges. inn. Med. **77**, (1971).

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